

# PLANT PHYSIOLOGY

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VOLUME 21

1946

THE SCIENCE PRESS PRINTING CO.,  
LANCASTER, PENNSYLVANIA



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### ERRATA

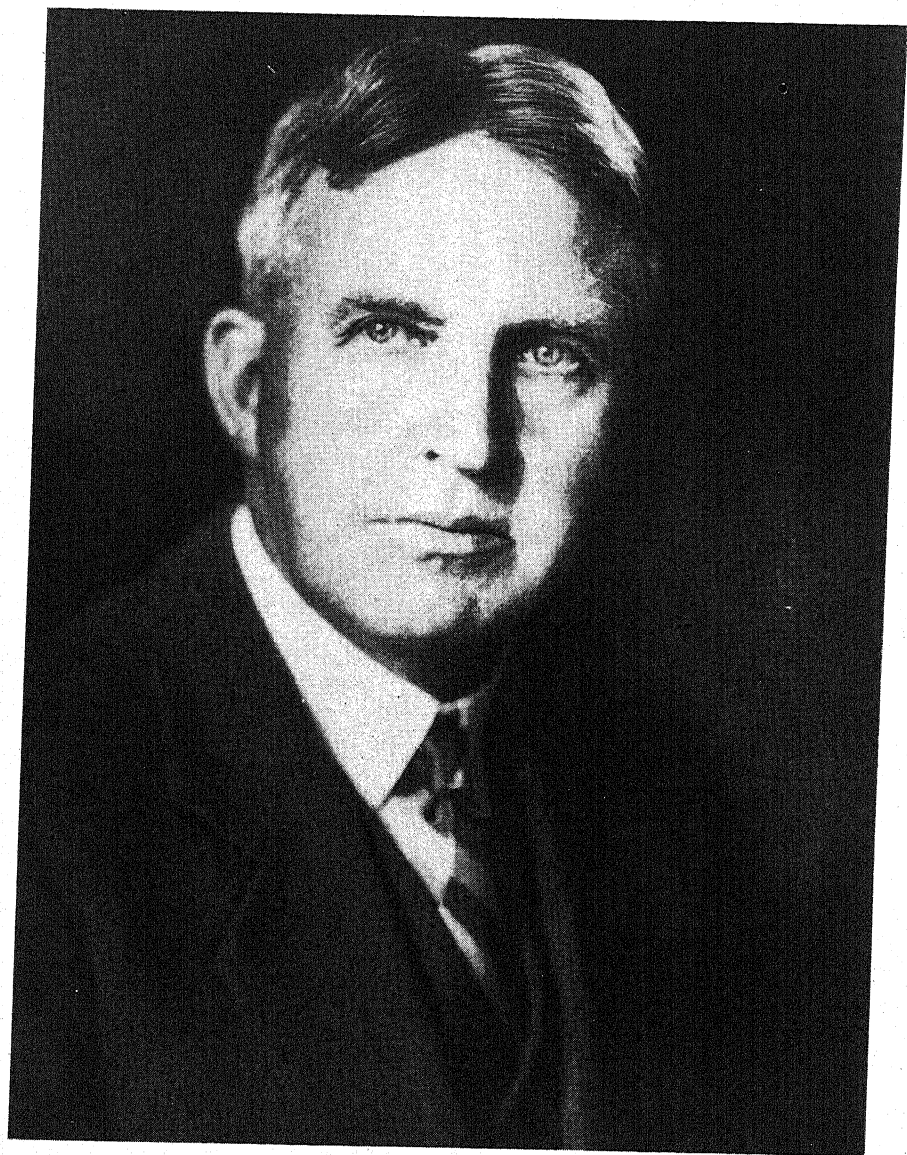
- Vol. 21, no. 1, cover, second line under contents, "Mycorrhizae" read Mycorrhizae.  
Page 4, line 30, "mitochondria" read mitochondria.  
Page 35, line 23, "to" read when.  
Page 87, figure 5, legend, "inorganic-nitrogen" read protein-nitrogen.  
Page 212, line 32, "*Adropogon*" read *Andropogon*.  
Vol. 20, page 163, no. 17, "Findlay, A." read \_\_\_\_\_; no. 18, "\_\_\_\_\_",  
read Findlay, A.  
Vol. 20, page 690, line 4 of table I, "unpuried" read unpurified.  
Vol. 20, page 689, line 9, "leaves" read leaves.  
Vol. 21, page 372, lines 1-2, "In 1915 a study . . . was published by MacDougal,  
Long, and Brown." It was Dr. Edmond Ray Long who collaborated in the  
study. Dr. Frances Long became associated with the Desert Laboratory in  
1917. The results of her efficient collaboration in the study of long-lived cells  
in the medullary tracts of cacti were published in 1927.

HOMER LEROY SHANTZ

IN CELEBRATION OF

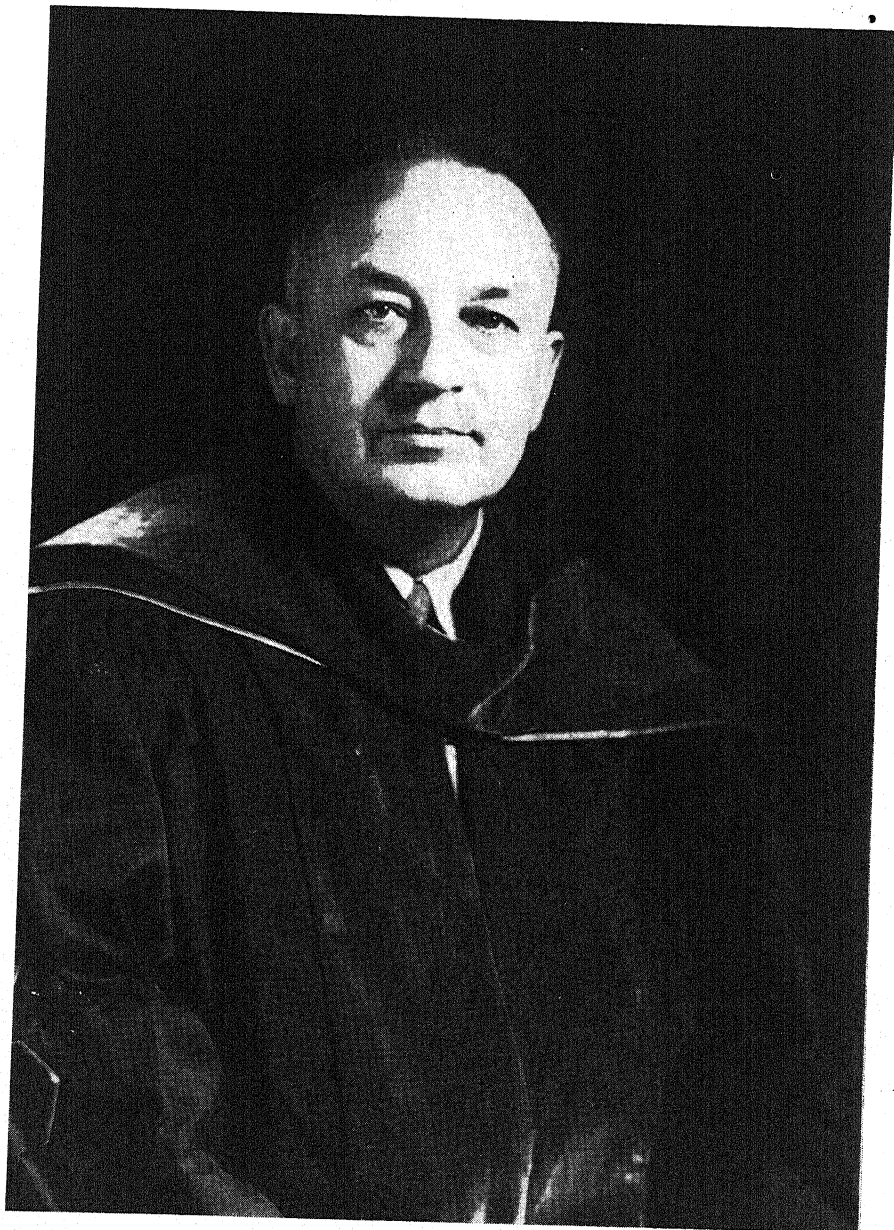
HIS SEVENTIETH BIRTHDAY

JANUARY 24, 1946









RODNEY BEECHER HARVEY  
MAY 26, 1890—NOVEMBER 4, 1945





## IN MEMORIAM

### RODNEY BEECHER HARVEY

MAY 26, 1890—Nov. 4, 1945

Sunday morning, November 4th, 1945, marked the passing of one of the outstanding Plant Physiologists of this generation. DR. RODNEY B. HARVEY, professor of Plant Physiology, Agricultural Botany, and Horticulture, died of a heart attack in the University of Minnesota Hospital after a very brief illness.

DR. RODNEY BEECHER HARVEY was born at Monroeville, Allen County, Indiana, May 26, 1890. He entered Purdue University in 1910, and was graduated in 1912. On June 17, 1916 DR. HARVEY married HELEN M. WHITTIER who survives him. Their five children are: HALE M. WHITTIER (deceased), RODNEY BRYCE, RHODA BEATRICE, HELEN ELIZABETH, AND ELEANOR W. HARVEY.

In 1912 DR. HARVEY was appointed Assistant Botanist at the large pharmaceutical manufacturing laboratory of the Eli Lilly Company of Indianapolis. In 1913 he entered the University of Michigan where the degree of Bachelor of Science was granted in February, 1915; he continued as a graduate student in Plant Physiology for the following semester. In June, 1915 he entered the University of Chicago as a candidate for the degree of Doctor of Philosophy.

In August of 1915, DR. HARVEY entered the U. S. Bureau of Chemistry as pharmacognocist, but was transferred within a month to work in Plant Physiology under DR. RODNEY H. TRUE in the Office of Plant Physiological and Fermentation Investigations in the Bureau of Plant Industry. His former professor, DR. WILLIAM CROCKER, came to this laboratory to do research for a year. During the period from 1915 to 1920 the influence of DRs. TRUE and CROCKER led HARVEY into research on the mineral nutrition of plants, salt absorption, permeability studies, enzyme activity, respiration, and winter hardiness. The broad field of research in progress in this laboratory thoroughly acquainted him with research methods and led him to publish on all of these special topics. DR. HARVEY also had the valuable friendship at this period of his career of DR. ERWIN F. SMITH and through this connection he undertook some researches in vegetable pathology, chiefly of a physiological nature. Under the influence of DR. TRUE he started a bibliographic index of his subject, which had been continued until his death, and now represents the most complete bibliography of its kind. Part of this compilation has been published in book form.

In 1917 DR. HARVEY returned to the University of Chicago as a Fellow, and continued research for the Ph. D. degree which was granted in 1918. His thesis was on the winter hardiness of plants. As a result of this work,

he introduced the Artificial Winter Test for determining the varietal hardiness of plants, a method which has been much used in northern countries by plant breeders to produce hardy plant varieties. Interest in this development was largely responsible for his invitation to join the staff of the University of Minnesota in 1920 where he became an outstanding research worker and authority upon this subject. He and the many students who studied with him published a great number of research findings on winter hardiness. Since 1931 he had been Professor of Plant Physiology, Agricultural Botany, and Horticulture.

DR. HARVEY's interest in research on fruits and vegetables came through his knowledge of the low temperature relations of plants and cold storage problems. Into the solution of these problems he threw all of his energy and experience and since 1924, many improvements in fruit processing have come from his laboratory. Ethylene ripening was made a subject of intensive research, leading to the demonstration that ethylene is produced naturally in fruits and vegetables during a process of ripening and blanching. The obnoxious flavors from spoiled fruit were found to be transferred to healthy fruits stored with them, and the use of cellulose wrappings and sterilization were found to decrease spoilage. The flavors of fruits depending upon minute quantities of esters and other such volatile compounds were found to be capable of manipulation, so that new flavors could be produced in stored fruit, or the flavors enhanced artificially. Considerable research was completed on the physiology of plant pigments and their transformations.

DR. HARVEY's range of interests extended over the whole field of plant physiological research. He developed research on the light intensities required by plants, and was the first one to grow plants from generation to generation entirely in artificial light. DR. HARVEY's demonstration of light requirements for the blooming of plants has led to widespread use of electric illumination in the study of plant reactions to light including the use of artificial light to cause the blooming of flowers out of their usual season, as well as in forcing seedlings for plant breeding purposes. In studies of seed germination DR. HARVEY made a most complete survey of the subjects of the length of the rest period of cereal seeds in relation to their storage conditions, methods of breaking dormancy in forest tree seeds, and the testing of seed viability.

In the practical applications of Plant Physiology in agriculture, DR. HARVEY developed a series of new chemicals for use in the control of weeds. He introduced the use of the cyanates, particularly sodium and ammonium sulfo cyanates, which are of value as fertilizers after their toxic action has been destroyed by bacterial decomposition in the soil, yielding nitrogenous fertilizers. Recently the xanthates were found to be of use as herbicides by his laboratory. He also found that persulfates and perchlorates had value as herbicides under certain conditions. At the time of his sudden death, DR. HARVEY was carrying on investigations of the effectiveness of certain hormones in weed eradication.

DR. HARVEY was one of the founders of the American Society of Plant Physiologists in 1924 and he served as its first secretary. In 1931 he became vice-president of the society, and was elected president in 1936. DR. HARVEY also was largely responsible for the formation of the Minnesota Academy of Science, an active organization which now numbers some five to six hundred members. In 1927-28 he was a fellow of the Guggenheim Foundation, studying at Cambridge University in the School of Botany with DR. F. F. BLACKMAN, and at the Low Temperature Research Station with SIR WILLIAM HARDY, PROFESSOR FRANKLIN KIDD, and PROFESSOR CYRIL WEST. He also studied at Bonn University with DR. ERNEST SCHAFFNIT, and at Leningrad with DR. NICOLA A. MAXIMOW. He was further commissioned by the Guggenheim Foundation to make a survey of research in progress in the Agricultural Experiment Stations throughout Russia.

For the year 1936-37 DR. HARVEY was director of the Florida Citrus Research Laboratory at Dunedin, Florida. This laboratory was founded to conduct research on fruit processing in connection with industrial and state institutions. In his industrial connections DR. HARVEY has been associated with The Food Machinery Company, Ind., the B. C. Skinner Company, Pacific Coast Distributors, and the United Fresh Fruit and Vegetable Association in connection with fruit processing; with the Carbide and Carbon Chemicals Company and the United Fruit Company in fruit ripening; with The American Potash Institute, the Kippers Coke Company, and the American Cyanamide Company in the development of herbicides; and with many other industrial concerns whose facilities have made possible the practical commercial development of the processes which he has developed. In 1942-43 he was director of the Division of Industrial Microbiology of General Mills of Minneapolis.

DR. HARVEY was the author of three books in the field of Plant Physiology, and of nearly two hundred bulletins and scientific papers. For many years he had the hobby of collecting historical materials relating to Plant Physiology and the History of Botany. He also had collected about 500 portraits of research men, laboratories, and student groups of historical interest. Included among the portraits were 67 paintings by the well-known Russian artist, STREBLOV, of life size portrait busts of plant physiologists.

At the time of his death, DR. HARVEY had just completed 25 years of service in plant physiology and related subjects at the University of Minnesota. In addition to his work as a teacher at the University and research worker in the Agricultural Experiment Station, he had perfected a large number of new methods for processing fresh fruits and vegetables which have been of great value to the industry and to the consuming public. Several of his processes had been patented, among which were the use of ethylene gas for hastening fruit ripening; enhancing the color of citrus fruits; x-ray methods of inspecting fruits and vegetables for internal defects and diseases; control of the flavor of fruits; fruit preservation and steriliza-

tion; and the blanching of citrus fruits to remove the green color from varieties that are of a greenish color when ripe. DR. HARVEY's process of ripening fruits by ethylene gas has had world wide use, having been applied to fruits such as bananas, tomatoes, honey dew melons, and tropical fruits which must be shipped in the green condition and ripened at destination. Practically all melons shipped from California to eastern ports are ripened with ethylene. The greater portion of the tomatoes shipped from California, Florida, and Mexico to the north in winter are ethylene treated. This process in combination with organic sterilizing agents and the use of cellophane wrappings and waxing processes has greatly improved the quality of fresh fruits and decreased spoilage from diseases incident to transport over long distances. DR. HARVEY had only recently perfected a process for waxing and polishing fruits which obviates the use of brushing and thus avoids injuries to the peel of soft fruits such as tomatoes, tangerines, pears, and apples. Bananas can be waxed and polished while in the bunch, to allow them to be transported over greater distances without shrinkage and spoilage. These processes have been a boon to the fruit and vegetable trade as well as to the consumer, and they have been used in many countries outside of the United States.

DR. HARVEY's work has been recognized in this country by the award of the honorary degree of Doctor of Science by Purdue University in June, 1939. He became a starred scientist in American Men of Science in 1933. He was also a corresponding member of the Botanical Society of Czechoslovakia and of the graduate faculty of the University of Madras, India. He was a fellow of the American Association for the Advancement of Science and a member of the following societies: The American Chemical Society, The Botanical Society of America, the American Pathological Society, American Society of Agronomy, American Society for Horticultural Science, American Society of Plant Physiologists, Minnesota Academy of Science, and the American Genetics Association. He belonged to the following fraternities: Sigma Xi, Gamma Alpha, Alpha Zeta, Phi Lambda Upsilon, and Gamma Sigma Delta.

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Vol. 21

Nb. 1

# PLANT PHYSIOLOGY

JANUARY, 1946

## CRITERIA OF NUTRITIVE RELATIONS OF FUNGI AND SEED-PLANTS IN MYCORRHIZAE

D. T. MACDOUGAL AND JEAN DUFRENOY

### Nutritive engagements of plants

The senior author has recently had occasion to scan nearly eight hundred abstracts of the contributions which constitute the main part of the literature of symbiosis in plants. This material was made available by the kindness of DR. A. P. KELLEY, the prospective author of a volume of Mycorrhizae in the New Series of Plant Science being produced by *Chronica Botanica*.

A very high proportion of the contributors base conclusions as to nutritive relations upon morphological characters, the results of germination tests, or aspects of life histories. It is becoming increasingly evident that plants anatomically engaged present examples ranging from destructive or pathogenic parasitism through intermediate stages of unbalance to stabilized symbiosis, further to associations incidentally or slightly beneficial to one or both elements; the advantages or disadvantages of the adherence of the two species are in some cases no more than mechanical support. In addition plants set free substances in the soil or substratum which may be toxic or beneficial to other species in proximity. It is obvious therefore that the category of terms currently applied to associations (facultative-obligate parasitism, commensalism, etc.) are without exact meaning, although of some convenience in text-books and general discussions.

The determining features of nutritive relations are those of the origination, flow, and ultimate disposal of material; the minor feature is that of the resultant alterations in tissue structure, cytological departures, and modifications of metabolism. It is therefore plainly evident that the nature of the association of two species may be determined with finality only on cytological and cytochemical evidence. Information included in the last category will be of value only if the main features of the chain of metabolic processes are adequately delineated. Thus, for example, in many cases of parasitism the invading organs of the parasite, generally with its vacuoles with a higher osmotic potential than those of the host, will cause the translocation of phosphorylated sugars to the cells of the host nearest the para-



site; some of this material will be drawn into the parasite, but a surplus may accumulate and be synthesized into starch. Concentration of material may result in renewed growth of the tissues of the host; this, with the surplus starch, may make it deceptively apparent that the presence of the fungus is beneficial to the host.

The authors have recently made a study of a terrestrial orchid with green leaves, a chlorophyllless orchid with no roots, and a pine tree, all with highly developed mycorrhizae features (6). The chain of metabolic processes and the compounds translocated from the fungus to the higher plants were found to be identical.

The soil fungi have an independent, non-measurable existence in the soil, and a minute fraction of their mycelia are engaged in the mycorrhizae. The material received by the higher plants is of great importance to their development and continued existence; full maturity may not be reached without such a supply, a condition applicable to probably more than nine-tenths of all seed plants. By the application of pigeon-holing, or of empirical terms mentioned above, this enormous number of species might be said to be parasitic on soil-fungi, which, however, would be simply a specious statement of the general inter-dependence of plants under natural conditions.

While the general features of symbiosis between fungi and seed plants as exemplified by mycorrhizae have been the subject of many contributions, no well-established conclusions have been reached as to the beneficial results of the association to the fungus partner. On the other hand the processes by which electrolytes and organic material are absorbed from the soil by mycelia, elaborated into compounds fundamental to protoplasm, and translocated to the higher plant are recognizable as described in later paragraphs.

Extended observations and contributions by other workers subsequent to our earlier paper make it possible to clarify some of the tentative conclusions advanced as to the synthesis and translocation of the auxins, carbohydrates, nucleo-proteins, phospholipids, etc., which constitute such important features of the chain of metabolic processes. The more nearly complete outline of the symbiotic relations presented appears to call for and to justify some speculation as to their origination.

In the engagement of fungi with the underground organs, roots, or stems, hyphae may form heavy felts on the surface, sending sparse branches into epidermal or cortical cells; or these branches may traverse the middle lamella so that a network encloses the protoplast. In any case, hyphal branches penetrate to the vacuoles amidst which they develop dense clumps or, in other types, large blasts at the ends of the hyphae. The stage at which translocation from the fungus to the cell of the higher plant occurs as well as the incidental cytological features vary widely.

The survival or behavior of the occupied cells—symbiosis or parasitism—is determined by the balance between the agencies which activate hydrogen from such metabolites as sugar and the agencies which activate atmospheric

oxygen so that the latter will accept the former. The activation of hydrogen or its transfer from the level occupied in sugar, for example, to a level at which it may be accepted by activated oxygen, depends upon dehydrogenases which comprise compounds of C, N, P, Fe, and S of high molecular weight and specialized structure, presenting active groups such as sulfhydryl. [See (9) for discussion of increased absorption of salts from soil, respiration, and fermentation in pine root segments.]

This dispersion of dehydrogenases entails the decompensation of aerobic respiration as evidenced by the progressive dehydrogenation of the phenolic compounds in solution in the vacuoles into quinone polymers.

So long as the dehydrogenases perform normally in aerobic respiration they re-hydrogenate phenolic compounds back to their original state as soon as they have released hydrogen to oxygen, thereby being dehydrogenated to quinoids. The continued ramification of hyphae may proceed in such a field. The dispersion of the dehydrogenases (complex compounds of C, N, P, and S) is, as noted, followed by the more complete oxidation and polymerization of the quinoids into gummy tannin masses with fixation of the proteins present. These visible masses constitute a barrier limiting the extension of the hyphal branches.

#### Cytochemical methods of analysis of nutritive relations of mycorrhizal elements

Chief interest centers in the arrangements of the structural constituents of the active cells of the cortex and especially the localization of the phosphorus complexes of the dehydrogenase system and the copper-protein systems acting as polyphenol oxidase in the derivation of the quinoids.

Valuable information may be obtained by vital staining to localize important cell components and by methods of fixation which give immediate reactions parallel to the changes which follow slowly a decompensated respiration in living cells.

#### VITAL STAINING

In the employment of vital stains it is important to note that the color reactions in the vacuolar solutions are of determinative value only so long as the vacuole is limited or enclosed by living cytoplasm.

When freehand sections of living pine mycorrhizae are immersed in a solution of neutral red, isotonic and isoionic to the vacuolar solutions, the polyphenols of the vacuoles of both the hyphae and cortex take on a bright red or purplish color.

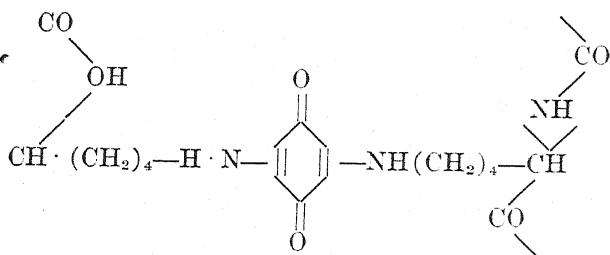
These polyphenols, following a decompensated respiration, oxidize to quinoids, developing reactive groups which unite with the proteins of the cytoplasm with a resultant fixation or killing, in a manner duplicating death from the action of formaldehyde (10).

Formaldehyde and quinone include an active carboxyl group. In acid solutions the aldehyde group of formaldehyde forms a methylol (hydroxymethyl) group with the amino groups of the protein.





In solutions between pH 5.5 and 7 the quinone penetrates proteins with slow oxidation, polymerization, and fixation of the polymer.



Reliable pictures of the spatial arrangement of cell constituents require, as a prerequisite to staining, fixation by a mixture of formaldehyde and potassium bichromate. As noted above, the formaldehyde supplies the carbonyl group which binds the proteins while the potassium bichromate brings out the "chromatin" effect in which the polyphenols are oxidized by the chromium salts to stable yellow and orange quinoid polymers.

After fixation as above the nucleoproteins in the prochromosomes, the resting nucleus, dividing nucleus, the chromosomes, mitochondria, and other plasts stain a deep black in iron haematoxylin while the cytoplasm remains a faint grey, and the phenolic compounds retain the yellow tinge of the previous chromatin reaction. Alternative to the above reactions, the nucleoproteins in the organs mentioned stain a deep red in acid fuchsin. A further differential reaction may be obtained by the addition of methyl green, which forms a complex with the acid fuchsin, the combination producing shades of purple and blue with the phenolic compounds.

Cells which have become filled with brownish masses of protein-polymerized complexes may be taken to be at the end of their activities in the nutritive system; although the nucleus may still retain an approximately normal structure and appearance as it does in the endodermis and pericycle.

The site of the high phenolase activity which finally results in the above conditions may be found by the use of aromatic amines such as phenylenediamine chlorhydrate, which in the presence of polyphenol oxidase yields a reddish quinoid which fixes the proteins in sections of living material.

#### UNMASKING PHOSPHORUS LINKAGES

The great importance of phosphorus in the metabolism of mycorrhizae is suggested by the fact that this element is to be found in compounds, as in ionic  $\text{PO}_4$  in vacuolar solutions, and in firmly bound complexes in liponucleo-proteins of the types characterizing mitochondria and other plasts. A variety of effects may be seen under the microscope after treatment with the well-known molybdenic reagent (6).

Water-soluble phosphorus compounds appear immediately as a blue phosphomolybdenic complex; after several hours the more firmly bound

phosphorus in the lipo-nucleo-proteins is unmasked. Mitochondria may be distinguished from other plasts by the further use of acid fuchsin, the specialized combinations in mitochondria becoming bright red while other plasts show a purple tinge. It is notable that while this particularized composition of mitochondria has been demonstrated, yet no well-supported conclusions as to the functions of these organs in the cell have been reached as noted below.

Phosphorus compounds are released in the dispersion of the dehydrogenases in tissues invaded by a parasite and flow toward the parasite. But in mycorrhizal roots of chlorophyllless plants, and in cultures of isolated segments of pine roots, all nutritive elements, not only P but also C, N, and S pass from the fungus associate to the root. Since the histological and cytological features of such roots do not differ in any discernible feature from those of the attached mycorrhizae of pines, it may be assumed that the nutritive relations are identical.

A cytochemical appraisal of the cortical cells of underground organs entering into symbiotic relationship with soil fungi, as above described, reveals the fact that C, H, N, O, P, and S, whether absorbed from the soil as electrolytes or as complex organic compounds, are metabolized into the higher groups, carbohydrates, phospholipids, proteins, etc., in the hyphae of the cooperating fungus, and are then translocated to the cortex of the higher plant.

In an earlier publication it was stated that the only carbohydrate recognizable in fungi was glycogen; since this substance was not diffusible it could not be regarded as the immediate source of glucose and starch which appears so abundantly in mycorrhizae. Phosphorylated glycogen, however, can be translocated as is glucose, and this compound may be regarded as the source of the carbohydrates in mycorrhizae, appearing as glucose and starch in the cortex.

#### PLASTIDS

The plastids show a special relation to certain features of phosphorus metabolism. The amyloplasts present in the cortex, but not in the fungus, synthesize starch from the phosphorylated carbohydrates received from the fungus in mycorrhizae or translocated from neighboring elements. Nucleo-proteids are built up in proteoplasts in both hyphae and cortex. Mitochondria, which are characterized by a rod-like form and a sharper molybdenic reaction than other plasts, occur in both hyphae and cortex. As noted above, the part played by these organs of the cell is obscure. It is possible that the reduction of their phosphorus content might coincide with their conversion into amyloplasts, a transformation still in controversy. That the number of these bodies in a cell decreases with maturity, and differentiation coincidental with the other plasts becoming more numerous, can be confirmed. The lateration in position of mitochondria in cells may yet furnish some clue to their cytochemical activity. These bodies are disposed

in strands or crowded around the small vacuoles in meristematic cells. In later stages, when the main vacuole occupies most of the volume of the enlarged cell, mitochondria are disposed at random in the thin peripheral layer of cytoplasm.

#### Diversity of morphological adjustments

While the chain of processes in which electrolytes and organic compounds are absorbed from the soil, elaborated into proteins, carbohydrates, and phospho-lipidic compounds, which are translocated to the tissues of the higher plant, conform to a general pattern common to all mycorrhizae, the anatomical engagement of the two symbionts shows the widest diversity.

The great majority of these cooperative associations are as the term implies, "fungus-roots"; in a large number of cases, however, the fungus enters the tissues of underground stems, as in many orchids, with modifications of structure carried to the extreme with loss of roots as in *Corallorhiza*.

The branches of the mycelium which penetrate the underground organs are barred from the stele by the formation of masses of tannin in the endodermis, and the hyphae extend apically in mature or nearly mature cells behind the meristematic region of tips of the roots or stems. In types of mycorrhizae in which the mycelium forms a felted mass around the roots or underground organs the enclosure may be complete and lasting as in *Monotropa*.

The main roots of pines, the stele of which is diarch, have a capacity for a high rate of elongation so that whitish terminals many centimeters in length are to be seen in the growing season. In a second stage, exfoliation of the cortex ensues, and a cambium layer originates in the pericycle with a subsequent growth in thickness much after the manner of stems. A characteristic morphogenic feature is the origination of myriads of lateral branches which attain a length of only a few millimeters from cell masses of the endodermis and pericycle and are monarch. These rootlets may originate in sterile soil and water cultures, and hence their origination may not be ascribed to the inciting action of the fungus with which they form mycorrhizae; a felted sheath of hyphae so nearly complete that only a very small area of the whitish tip of the rootlet remains visible. The hyphal branches which penetrate the cortex extend internally to the endodermal barrier and apically to the minute mass of embryonic cells at the tip. The entire arrangement comes to an end with the season, and a new set of mycorrhizae may be developed in the next season. The presence of the fungus is not necessary for the germination of pine seeds, and the plantlet can carry on normal development for two, perhaps three, seasons without mycorrhizae. Conditions adverse to the growth of the soil-fungus may result in seasons in which no mycorrhizae are formed with consequent impairment of the nutrition of adult trees.

Similar seasonal conditions have led to contradictory conclusions by observers, some of whom have examined individual plants with mycorrhizae,

while others have encountered individuals under conditions in which the cooperative arrangement was not completed due to adverse soil conditions.

Close and continuous engagement of the two symbionts, including the entire life of the seed-plant, is to be seen in some cases. The presence and action of compounds elaborated by the fungus may be necessary for the germination of the seeds and growth to maturity as exemplified by *Corallorhiza*. The mycelium in the cortex which keeps pace with the extension by growth of the rhizomatous stem sends hyphal branches *outwardly* into the soil simulating root hairs. The fungus forms a mycelium in the cortex of the internodes of *Aplectrum* which it enters through trichomes near the base, and the presence of the fungus is in transit toward the apex from which entrance in the roots of the next internode is made. Hyphal branches pass outwardly through the root hairs into the soil in this case. As has been previously described, unknown factors may cause unusual activity in the mycelium in its passage through the internodes with resultant development of coralloid branches. Numberless different types of engagement of the two symbionts might be cited, but the nutritive relations of the two plants concerned can be reliably determined only by a cytochemical analysis of the flow of material.

#### Genetical considerations

No conclusive evidence as to the manner in which characteristic morphogenic alterations, some of which are of wide scope, were initiated and became transmissible by seeds is available. Vegetative mutations in which underground stems (offsets) of *Aplectrum* respond to penetration of hyphae by the formation of mycorrhiza branches identical in general features with the underground coralloid stems of the nearly related *Corallorhiza* have been previously described (6). Such mutations occur numerously and in widely separated habitats but may be induced culturally and are not transmissible by seeds. The transformation is firmly fixed in *Corallorhiza* and the mutation affects the system of genes to such an extent that the capacity for the formation of roots has been lost entirely. Reduction of foliar surfaces and of chlorophyll, with accompanying modifications of the transpiratory mechanism, are very marked.

Attention is naturally directed to the minute seeds of these orchids and to the part which the symbiotic fungus may play in their germination. Cultural tests of a number of species of terrestrial orchids show that the seeds of some are activated only in the presence of the symbiotic fungus, others in water, and others in nutrient solutions to which some form of sugar, levulose, dextrose, or sucrose has been added (1).

In the case of *Corallorhiza innata*, which offers all of the morphological and physiological features of *C. maculata*, DOWNIE (2, 3, 4) found that "the seed of this orchid will not germinate in water nor in sugar solutions to which plant extracts have not been added," and it may safely be assumed that *C. maculata* also requires the presence and action of substances derived from the fungus for the awakening of the embryo.

The minute seeds of this plant consist of a pear-shaped embryo about 0.02 mm. in length and a thickness of 0.01 mm. in the larger part, enclosed in a fragile transparent testa. This delicate structure would appear to be singularly vulnerable to environmental agencies, but such seeds have been seen to lie in moist humus for many months with comparatively few fatalities. It is in this condition that hyphae of the symbiotic fungus may be seen to enter the cells of the smaller end of the embryo and form coils or pelotons in the neighborhood of the nuclei, and some of the outer cells of the suspensor elongate to form absorbing trichomes similar to those of the adult rhizomes. Development of the embryo of *C. innata* was such a low rate that its length increased only eight times in nine months.

Germination of seeds of *C. maculata* showed a similar low rate. The contents of several pods placed in a layer of moist soil from the habitat in October, 1944, presented but little change until late in May, 1945; at this time a few embryos had begun enlargement as observed with a magnification of 80. Most of the testas were still intact; some hyphae penetrating the coats to the embryos were seen. The identity of the cytochemical processes of the orchid mycorrhizae with those of the pines may justifiably be extended to include accelerated absorption, respiration, and metabolism as described by ROUTIEN and DAWSON (9).

The uptake of oxygen in mycorrhizal roots was two to four times that of non-mycorrhizal members, with consequent increase of energy available for carrying nutrient substances across membranes. Anaerobic respiration was also increased with implied greater production of carbon dioxide and an increased supply of potential hydrogen ions.

It would be reasonable to attribute augmented possibilities of genetical modifications in young mycorrhizal protocorms with many metabolic processes being carried at unusual high levels. Compounds representing the main components of the cells are formed in the hyphae including a carbohydrate, glycogen, which non-diffusible, becomes so when phosphorylated and after translocation appears as glucose in cortical cells where synthesis of starch takes place.

The translocation of acid-soluble, mono-nucleotides to the cells of the embryo would disturb the balance in the cytoplasm with the desoxy-, highly-polymerized, ribose nucleotides of the chromosomes, while the introduction of any foreign proteins might have a disturbing effect. It is also to be said that the addition of phosphorous complexes which would enter into competition with those normally playing a part in the respiratory systems might be a factor in inducting genetical departures.

Whatever the nature of the cytochemical action, the genes responsible for the origination and production of roots in *Corallorhiza* have been cancelled or masked so that no rudiments of these organs are to be seen in any stage of the development of the vegetative axis. Reduction of the photosynthetic mechanism to a variable near the vanishing point has also occurred.



Concomitant with the losses or degradations mentioned, the overall system of metabolism has undergone adjustments. In addition to the reception of auxins, hormones, etc., from the associated fungus all of the materials entering into flower, fruit, and seed formation may be produced in the mycorrhizal rhizomes and translocated apically to the inflorescence as described by MACDOUGAL and REED (7, 8).

No evidence to the effect that the association of a fungus with its mycorrhizal partner is necessary for its continued existence is yet available. Neither has any determination of possible alterations been made. The results of cultural experiments with other microorganisms suggests the possibility that the nutritive conditions furnished by cortical cells might induce the development of specialized strains of the fungus not easily separable from the main mass of the mycelium.

### Summary

1. The nutritive relations of associated plants may vary by minute gradations from pathogenic parasitism through symbiosis to effects purely mechanical and incidental.
2. The main components of protoplasm may be elaborated by the fungi of orchid and pine mycorrhizae and translocated to the cortex.
3. The reaction of cells of the higher plant entered by fungi is determined by the balance between the agencies which activate hydrogen and those which activate oxygen.
4. Decompensated respiration, especially in cells of the pericycle and endodermis, results in polymerization of the quinoids into gummy tannin masses, the presence of which forms a barrier to the extension of hyphae.
5. Technique of cytochemical analysis of nutritive relations is described.
6. The minute seeds of terrestrial orchids present many possibilities of genetical modification which might result from the action of environic factors and especially of substances received from associated fungi which enter the embryo at an early stage. Accelerated metabolism and the translocation of foreign nucleoproteids and of phosphorous complexes are of special significance. The principal mutations include loss of roots, reductions or total loss of photosynthetic mechanisms, and the acquisition of a capacity to mature seeds in the absence of photosynthesis.

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GROWTH STIMULATION AND PHOSPHORUS ABSORPTION OF  
MYCORRHIZAL AND NON-MYCORRHIZAL NORTHERN  
WHITE PINE AND DOUGLAS FIR SEEDLINGS  
IN RELATION TO FERTILIZER  
TREATMENT<sup>1</sup>

A. L. McCOMB AND JOHN E. GRIFFITH

(WITH FOUR FIGURES)

Although there is no longer much question of the dependence of pines and many conifers on fungal symbionts forming mycorrhizae, there is considerable disagreement concerning the manner in which this symbiosis stimulates the growth of trees. A marked change and enlargement of the tree seedling root system follows mycorrhiza formation, and according to various authors the fungus partner makes available to the tree organic nitrogen (6), carbohydrates (4, 11), water (1), nutrients (2, 4, 5, 10), auxins and enzymes (3, 4, 8), and in addition destroys toxic soil compounds resulting from certain types of organic decomposition (7, 8).

In a recent paper one of the authors (5) described a case of the dependence of pines grown on prairie soil on mycorrhizal symbionts, showing the importance of phosphorus and suggesting that growth stimulation was due to increased respiratory activity associated with higher levels of phosphorus and with growth stimulators derived from the fungus. ROUTIEN and DAWSON (10) have shown increased salt absorption associated with the heightened oxygen intake and carbonic acid production of mycorrhizal *Pinus echinata*. MACDOUGAL and DUFRENOY (4) indicate that mycorrhizal fungi supply phosphorus and other nutrients, carbohydrates, auxins, and B vitamins and suggest that much of the increased activity of mycorrhizal roots is keyed to the metabolism of phosphorus.

This investigation was concerned with the growth and phosphorus absorption of two conifers on inoculated and uninoculated soil given several fertilizer treatments. Its primary object was to determine what combination of phosphorus application and/or soil inoculation produced best development and how phosphorus absorption was affected.

### Experimental

This was a field experiment carried out in seed beds on O'Neill sandy loam at the State Forest Nursery, Ames, Iowa. Two adjacent beds were selected on soil which had never grown conifers before, and which previous experience had shown did not contain active mycorrhizal fungi. One bed was treated at the rate of one bushel per 400 sq. ft. with coniferous duff and humus known to contain active mycorrhizal formers. This bed and the other were divided into three replicates for each species and individual random-

<sup>1</sup> Journal Paper no. J-1308 of the Iowa Agricultural Experiment Station, Project no. 612.



ized plots treated with one of six phosphorus or phosphorus nitrogen and potassium combinations. The treatments were as follows:

	POUNDS PER ACRE OF FERTILIZER IN TREATMENTS						
	TREATMENTS						
	1	2	3	4	5	6	7
	lb.	lb.	lb.	lb.	lb.	lb.	lb.
20% superphosphate .....	.....	500	1000	1500	1000	1000	1000
Ammonium sulfate .....	.....	.....	.....	.....	150	300	150
Potassium sulfate .....	.....	.....	.....	.....	.....	.....	300

Part of each bed was seeded in June, 1940, to northern white pine (*Pinus strobus* L.) and the other part to Douglas fir (*Pseudotsuga taxifolia* (La Marek) Britt.). In May, 1941, the beds were thinned to reduce competition effects, and plots receiving nitrogen were refertilized with ammonium sulphate. During the fall of 1941 the seedlings were removed, twenty seedlings being selected mechanically from each plot for growth measurements and study of the root systems, the remainder dried and ground for analysis. Phosphorus was determined by igniting 1 gram ground tissue with  $\text{Mg}(\text{NO}_3)_2$  and  $\text{HNO}_3$ , taking up residue in  $\text{H}_2\text{SO}_4$ , diluting, and determining colorimetrically as ammonium-phospho-molybdate.

Two-year-old white pine seedlings (fig. 1) made satisfactory growth on all plots except the uninoculated controls. There was no significant difference in weight or height among any of the inoculated or any of the uninoculated fertilized plots. Only the uninoculated, unfertilized controls (fig. 2) were significantly different from the others, and this difference corresponded to a much lower tissue phosphorus content and to a marked reduction in the number of short roots. No significant difference associated with nitrogen or potassium fertilization was noted.

Examination of the short roots of white pine showed three classes to be present (fig. 3): (1) hypertrophied with a macroscopically visible, external fungal mantle and with inter- and intra-cellular hyphae; (2) hypertrophied with a tight, compact, mantle, microscopically visible only on sectioning and with intercellular hyphae only; and (3) roots not hypertrophied nor mycorrhizal. All of the short roots of seedlings on the uninoculated, unfertilized plots were non-mycorrhizal (class 3). Short roots of seedlings from inoculated plots regardless of fertilizer treatment were of all three classes, while roots from uninoculated fertilized plots were chiefly of classes 2 and 3. The most significant points in the white pine data appear to be that phosphorus fertilization induced the formation of mycorrhizae in uninoculated plots, and that without either inoculation or fertilization, both of which resulted in mycorrhizae, the seedlings eventually died.

Douglas fir seedlings (fig. 4) made satisfactory growth on inoculated plots only. On uninoculated plots growth increased with increase of phos-

phorus application, but in no case was fertilization equivalent to inoculation. Seedlings from uninoculated, unfertilized plots were dying at the end of the second growing season. Nitrogen and nitrogen and potassium, both in

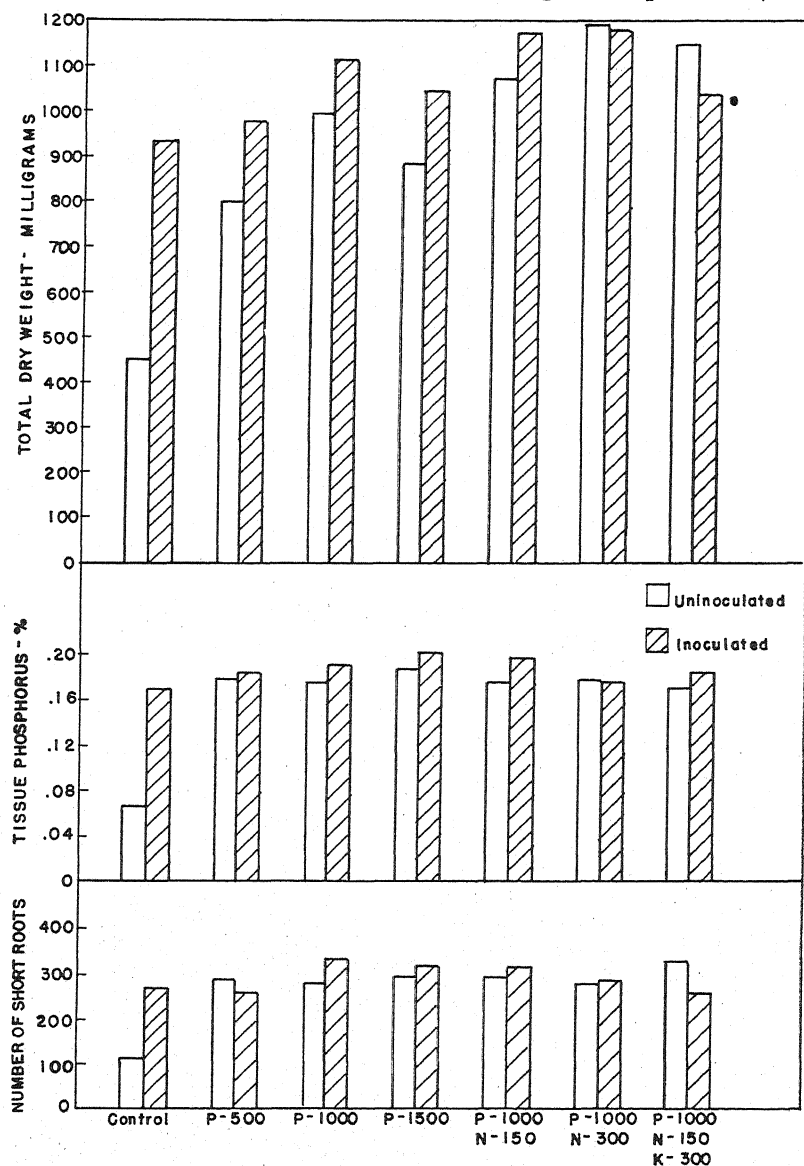


FIG. 1. Dry weight, percentage of phosphorus in tissue, and number of short roots of northern white pine on inoculated and uninoculated O'Neill soil treated with various fertilizers.

combination with phosphorus, caused no significant growth increase over that obtained using phosphorus alone. The most significant points in these data are the marked superiority in growth of seedlings from inoculated soil

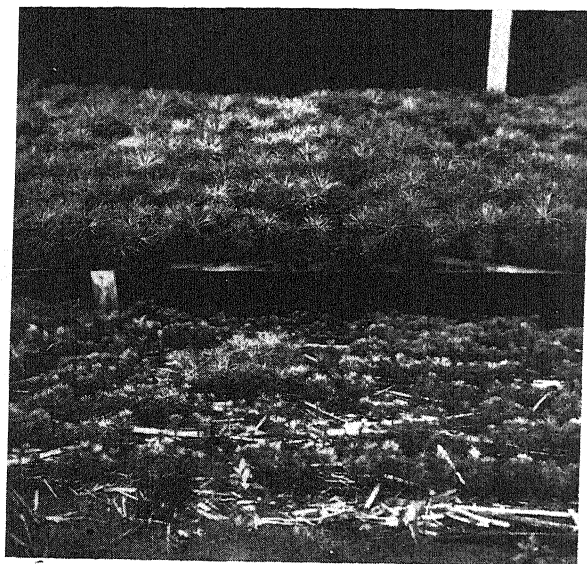


FIG. 2. Two-year-old white pine seedlings on unfertilized O'Neill soil. Inoculated (above) and uninoculated (below). Note small, fortuitously inoculated seedling clump in left center of uninoculated plot.

and the three-way parallelism on uninoculated plots among dry weight or height, phosphorus content of tissue, and number of short roots.

Short roots of Douglas fir were of two classes: (1) those hypertrophied but with no fungal mantle externally visible with the microscope and (2) non-hypertrophied. Sectioning showed that roots lacking hypertrophy were non-mycorrhizal. Hypertrophied roots from inoculated plots were mycorrhizal and from uninoculated plots non-mycorrhizal, although the number of roots studied was insufficient to draw absolute conclusions. The number

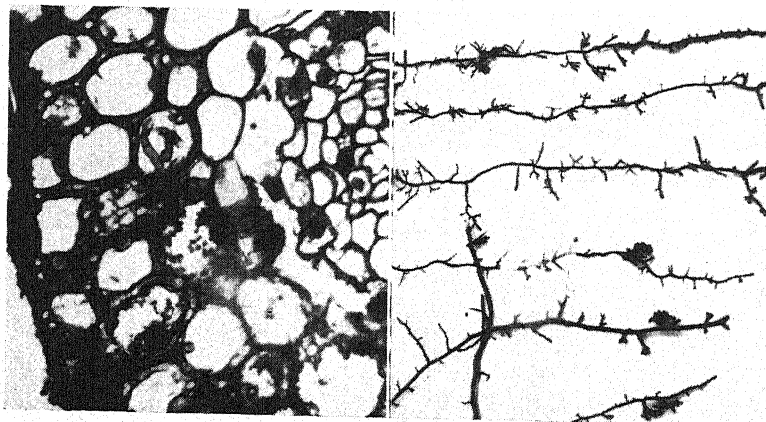


FIG. 3. White pine mycorrhizae. Left, section showing inter- and intra-cellular hyphae (325 $\times$ ); right, root branches with mycorrhizae. Coraloid clumps below are class 1; other hypertrophied shoot roots are class 2.

of hypertrophied short roots on uninoculated plots increased with phosphorus application from 7 per average seedling in the control to 31 for the 1500-pound superphosphate application. Thus, as contrasted to the white

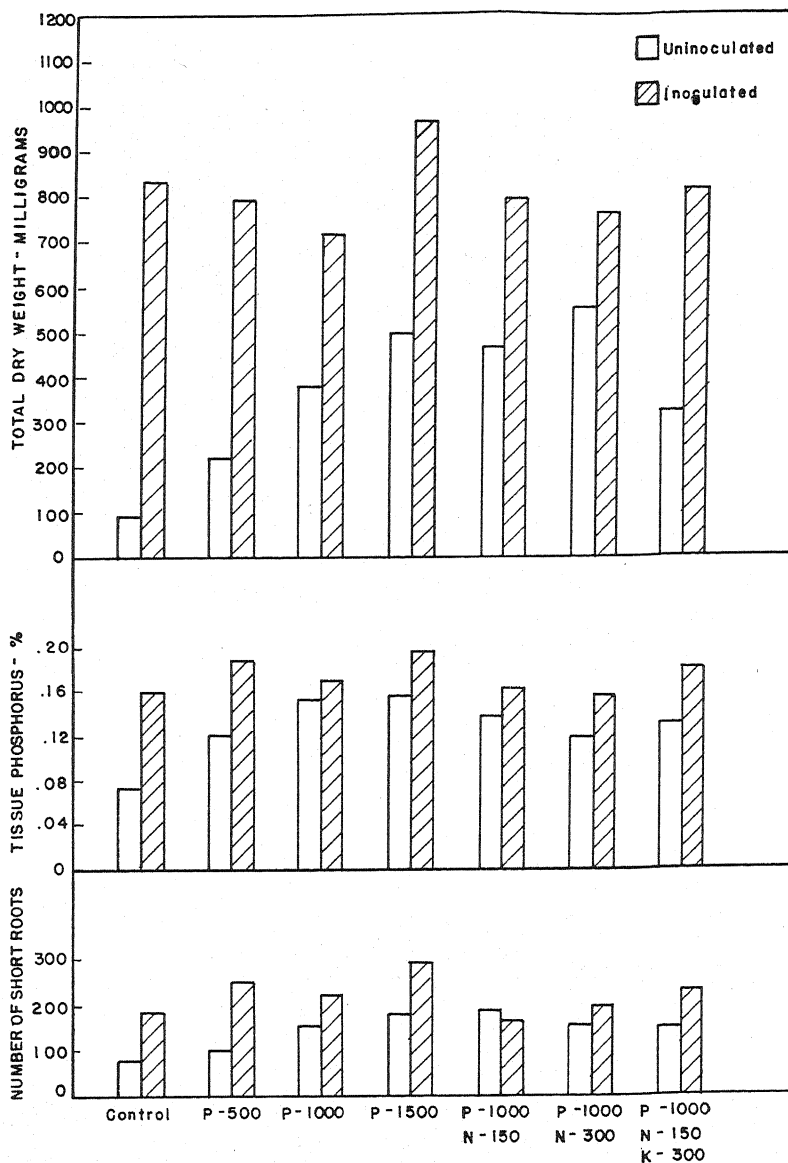


FIG. 4. Dry weight, percentage of phosphorus in tissue, and number of short roots of Douglas fir on inoculated and uninoculated O'Neill soil treated with various fertilizers.

pine, phosphorus fertilization of Douglas fir on uninoculated soil did not result in mycorrhizae formation even though some root stimulation took place.

Considering the data for both species the growth stimulation due to straight soil inoculation followed by mycorrhizae formation is outstanding. The formation of mycorrhizae on fertilized, uninoculated plots of white pine and the difference in structure of these mycorrhizae, as contrasted with some of those forming on seedlings from inoculated plots, suggests that there was present in the soil a fungus capable of forming mycorrhizae and stimulating the seedlings when either or both it and the seedling were first stimulated by phosphorus. No such relationship obtained with Douglas fir.

In general, the phosphorus content of the seedlings was correlated with growth. This is most strikingly illustrated with Douglas fir where tissue phosphorus concentrations of 0.16 per cent. appeared adequate for maximum growth. Non-mycorrhizal Douglas fir seedlings on uninoculated plots grew faster and absorbed increasing quantities of phosphorus as the rate of phosphorus application increased, *but, although tissue phosphorus concentrations reached levels producing adequate growth of mycorrhizal seedlings on inoculated plots, growth of non-mycorrhizal seedlings on uninoculated plots was significantly inferior.* Since total tissue phosphorus was adequate for good growth and since no significant response has been obtained with N, K, or Ca, the obvious conclusion is that the seedlings were receiving additional stimulators from the mycorrhizal association. These data all indicate that the most important rôle of mycorrhizal fungi is that of stimulating the metabolic processes of the roots and that this stimulation, as has been suggested by the authors (5) and MACDOUGAL and DUFRENOY (4), is associated with the rôle of phosphorus and other stimulators in root respiration and metabolism.

### Summary

Two-year-old seedlings of northern white pine and Douglas fir made very satisfactory growth on O'Neill soil inoculated with coniferous humus containing mycorrhizal fungi. On uninoculated soil fertilized with phosphorus, white pine seedlings formed mycorrhizae and made satisfactory growth; but Douglas fir seedlings, although responding moderately to phosphorus fertilization, did not form mycorrhizae nor maintain normal growth rates. Excepting uninoculated Douglas fir, good growth in every instance was associated with high phosphorus absorption. The fact that Douglas fir made poorer growth on uninoculated, fertilized plots than on inoculated plots, even though seedling phosphorus levels were high, and no response was being obtained from nitrogen and potassium, suggests a mycorrhizal stimulating effect of mycorrhizal fungi on conifer seedlings is due to heightened metabolism, associated in this instance with transfer of phosphorus and growth stimulators from fungus to seedling.

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## EFFECT OF VARIOUS OXYGEN AND CARBON DIOXIDE CONCENTRATIONS ON COTTON ROOT DEVELOPMENT<sup>1</sup>

O. A. LEONARD AND J. A. PINCKARD

(WITH NINE FIGURES)

### Introduction

Under field conditions, the concentrations of oxygen and carbon dioxide at the surface of cotton roots are thought to be extremely variable. Gross analyses made by LEONARD (10) show the percentage of oxygen in the soil air under cotton to vary from 0 to 21 for Houston clay, from 10 to 21 for Sarpy fine sandy loam, and from 18 to 21 per cent. for Ruston sandy loam. While these values only approximate effective concentrations at root-gas interfaces, they do indicate in a relative way the conditions of aeration within different soil types. Although the differences in behavior of cotton on these different types of soil may be attributed to differences of particle size, fertility, water movement, and numerous other variables, the rôle of aeration appears to be quite important. Unpublished data of the present writers indicate that aeration and mechanical composition of the soil may prove to be an important factor in the distribution of the *Fusarium* wilt disease of cotton. The importance of soil aeration as it applies to the culture of plants in general is well appreciated, although many of the details are yet to be investigated.

The purpose of the present study was to determine the effect of carbon dioxide and oxygen mixtures on the growth and development of cotton roots in nutrient solutions. The study seemed necessary in order to obtain data for the proper interpretation of the behavior of cotton on different soil types with and without the presence of the *Fusarium* wilt disease.

### Methods and materials

The general plan of the apparatus employed for subjecting the roots of seedling cotton plants to stated concentrations of oxygen and carbon dioxide alone and in mixtures with nitrogen is illustrated in figure 1. By means of a siphon arrangement, the gas in container B was forced through a nutrient solution in which the plants were supported. Although only one culture tube and one plant is illustrated in figure 1, two tubes were used, each containing two plants. A battery of several such units was used, each provided with a different concentration of gas. The entire equipment was operated before a large laboratory window or in the greenhouse. Supplementary illumination was provided by a fluorescent lamp which was operated continuously. In some experiments (not reported) no supplementary illumination was used; in others, artificial light supplemented daylight sufficiently

<sup>1</sup> Published with the approval of the Director, Mississippi Agricultural Experiment Station, Journal Paper no. 97, new series.

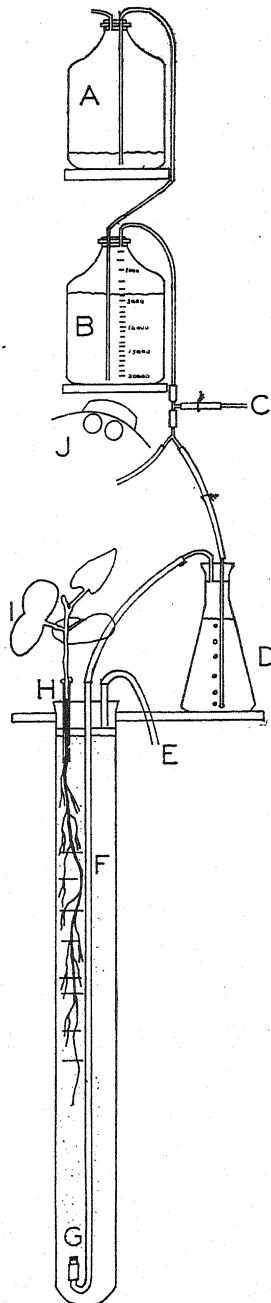


FIG. 1. A diagrammatic view of the apparatus used in aeration experiments. A, five-gallon bottle; B, five-gallon bottle calibrated to hold 20 liters of air at atmospheric pressure; C, air inlet for making gas mixtures; D, Erlenmeyer flask holding reserve nutrient solution; E, gas escape tube; F, culture tube; G, gas outlet; H, glass tube in which cotton plant was inserted; I, cotton plant; J, fluorescent light.



to make a photoperiod of 15 hours. The relationship between the percentage of oxygen and carbon dioxide and cotton root growth was similar to that obtained with continuous illumination. The culture tubes were placed in a darkened air- or water-bath in which the temperature was maintained at approximately 30° C. in some experiments and 28° C. in others. Air temperatures about the shoots were not controlled.

Compressed cylinder gases were either used directly or further purified by appropriate methods. They were introduced into the system through opening C and were utilized from container B (fig. 1). Approximately 40 liters of gas was passed through each pair of culture tubes in 24 hours. To prepare a given concentration of mixed gases, distilled water was forced from calibrated container B. The composition of the mixtures were determined at frequent intervals by a portable gas analysis apparatus. Oxygen concentrations were easily prepared accurately, although carbon dioxide concentrations frequently varied over a range not exceeding 10 per cent. of the required concentration. Dissolved oxygen in the culture solution was determined by the Winkler method (1).

A modified Knop's solution of approximately the same composition shown by LOOMIS and SHULL (12) was used in the culture tubes. In tests in which nitrogen was supplied in the ammonium form, ammonium chloride and calcium chloride replaced an equivalent quantity of calcium nitrate. The initial pH of the nutrient solution was adjusted at 5.4 to 5.6 using a weak solution of potassium hydroxide, the exact pH varying somewhat with different experiments. The nutrient solutions containing nitrates usually shifted to a pH of about 6.0 and the solutions containing ammonia to a pH of about 4.8 before being changed. If the solutions containing ammonia become too acid (pH of 4.0 or less) root growth ceased. The pH of solutions receiving high concentrations of carbon dioxide were stabilized by using a buffer composed of potassium acid phosphate and potassium hydroxide. The solutions were prepared from distilled water and most of them were changed at intervals of not more than three or four days. Solutions saturated with pure gases were not changed during the course of an experiment.

Cotton seeds of the Stoneville 2B variety were acid delinted, dusted with a mercurial disinfectant, and placed between moist paper towels for germination. If no seed disinfectant was used, a low yield of healthy seedlings resulted, even though all glassware, solutions, and apparatus were boiled or autoclaved before use. After the seeds germinated, the seed coats were removed with sterile forceps and the seedlings supported in tap water on a paraffin-impregnated cloth. After the cotyledons were expanded, healthy seedlings were selected, and the radicals were inserted through short glass tubes held in no. 7 rubber stoppers. A paraffin-petroleum-cotton-lint sealing compound held the seedlings in place and prevented exchange of gases through the openings after the stoppers were placed in the culture tubes. Air was passed through the culture solution for two or three days to permit

the seedlings to become established. The air or gas was released through a sintered glass plate in the bottom of the 24-inch culture tubes so as to break up the gas bubbles.

Growth of the seedling roots was determined by measuring the length of the tap root at 24-hour intervals. The experiments were terminated 14 to 16 days after the gases were first introduced. Notes were made on the

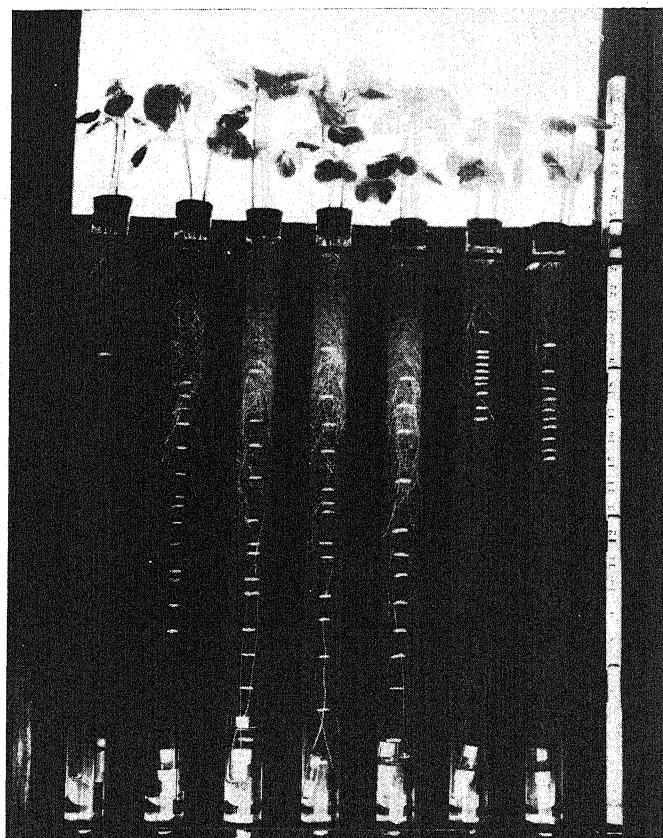


FIG. 2. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 13). Marks on the tubes represent tap-root elongation at 24-hour intervals. The gas mixtures from left to right were (percentages):

O <sub>2</sub>	0	5	10	15	21	90	100
CO <sub>2</sub>	10	10	10	10	10	10	0

appearance of the seedlings, and the fresh and dry weights of the shoots and roots were recorded.

A total of 20 separate experiments were conducted over a period of one and one-half years; although only seven of these experiments are actually referred to in the text, the results from the other experiments were similar to the ones reported.

## Results

## OXYGEN REQUIREMENTS

The optimum oxygen concentration for cotton root development was thought to depend upon a certain range of carbon dioxide concentration. To determine this range, oxygen, carbon dioxide, and nitrogen were combined in the proper proportion to form the desired mixtures. Oxygen was

TABLE I

APPEARANCE OF 14-DAY-OLD COTTON SEEDLINGS GROWN IN LIQUID CULTURES SATURATED WITH CARBON DIOXIDE AND OXYGEN AT STATED CONCENTRATIONS AND WITH AMOUNT OF DISSOLVED OXYGEN IN THE CULTURES. RESULTS FROM SEVERAL EXPERIMENTS SUMMARIZED

GASES USED		DISSOLVED OXYGEN IN CULTURES*		APPEARANCE OF TOPS	APPEARANCE OF ROOTS
CO <sub>2</sub>	O <sub>2</sub>	WITH PLANTS	WITH-OUT PLANTS		
%	%	p.p.m.	p.p.m.		
None	None	0.0	0.0	Little plants, small green leaves	Roots healthy, no growth. Branch roots developed if transferred to water saturated with air
10	None	0.0	0.0	Little plants, dark green leaves	Do.
10	0.5	Trace	0.1	Do.	Do.
10	1.0	0.3	0.4	Little plants, green leaves	Roots healthy, trace of growth
10	1.5	0.5	0.6	Medium plants, green leaves	Roots healthy, thick, some growth
10	2.5	0.8	1.0	Large plants, green leaves	Roots healthy, medium thick, good growth
10	5.0	1.8	2.0	Do.	Roots healthy, thin, very good growth
10	7.5	2.7	3.0	Do.	Do.
10	10.0	3.6	3.8	Do.	Do.
10	15.0	5.4	5.8	Do.	Do.
10	21.0	7.0	.....	Do.	Do.
10	90.0	33.0	.....	Medium plants, green leaves	Roots frequently unhealthy and parasitized, poor growth. Healthy roots produced if transferred to water saturated with air
None	100.0	37.0	.....	Do.	Do.

\* Temperature of 28° C.

held constant at 21 per cent. while carbon dioxide and nitrogen were varied. Considerable root growth occurred up to 30 per cent. carbon dioxide while vegetative growth appeared to be excellent and unaffected over the same range. As a result of these tests, 10 per cent. carbon dioxide was selected as a constant with which to compare different concentrations of oxygen.

The apparent optimum concentration of oxygen for seedling root elongation, with carbon dioxide held constant at 10 per cent. was found to range

between 7.5 and 21 per cent. under the conditions described. Figure 2 illustrates a typical experiment in this series, and table I summarizes the results of all oxygen concentrations investigated. Figure 3 illustrates the variation occurring in three selected experiments.

Daily elongation of the tap roots is graphically illustrated in figure 3. During the first two weeks of seedling growth, tap-root elongation appeared to progress at a uniformly high rate. The greatest elongation for any one day was 67 mm.; it occurred at 21 per cent. oxygen and 10 per cent. carbon dioxide. Variation in root temperatures slightly above 30° C. sharply restricted the daily rate of tap-root elongation. Any prolonged reduction in intensity of illumination also limited root elongation, but the effect was not apparent for two or three days. Experiment 15 was conducted at light intensities somewhat lower than that of Experiment 17 (fig. 3). Although

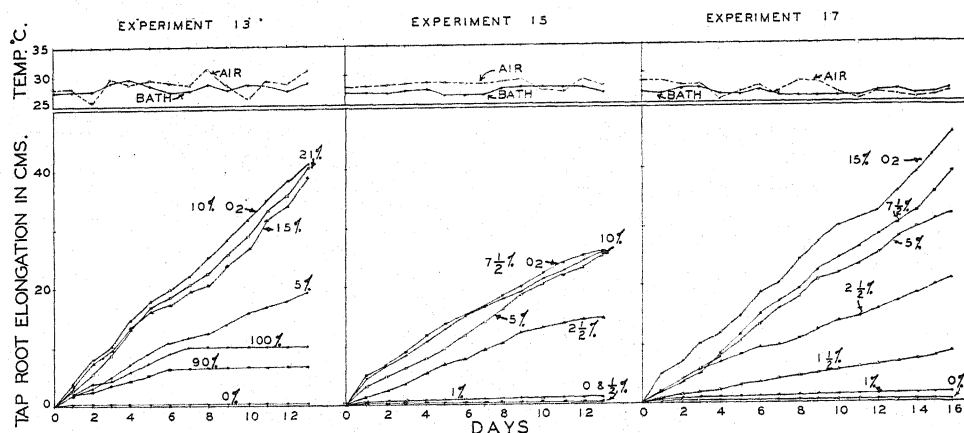


FIG. 3. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots. Carbon dioxide was held constant at 10 per cent., except at zero per cent. oxygen in Experiments 13 and 17 and at 100 per cent. oxygen in Experiment 13.

two plants were grown in the same tube and duplicate tubes used, the daily rate of elongation for each plant varied widely at times. No relation could be observed between stage of leaf development or leaf area and tap-root elongation.

The minimum concentration of oxygen tolerated by roots appeared to lie below one-half per cent. (figs. 2, 4) and zero per cent. (fig. 5). No root growth occurred under these conditions. The roots remained white and did not appear to be injured by anaerobic conditions. If air was substituted for the mixture containing zero oxygen, lateral roots were promptly initiated, and some of the old branch roots began to grow again. The tops of the plants were a darker green (when carbon dioxide was held at 10 per cent.) than those of plants receiving higher concentrations of oxygen.

At maximum concentrations of oxygen (90 and 100 per cent.), both root and top growth were restricted (fig. 2), but the color of the leaves was not affected as at low oxygen concentrations. If air replaced the gas mixtures

of high oxygen concentrations, lateral root development was initiated, indicating that the pericycle was not permanently damaged. Microorganisms attacked the roots vigorously at high oxygen concentrations. Although

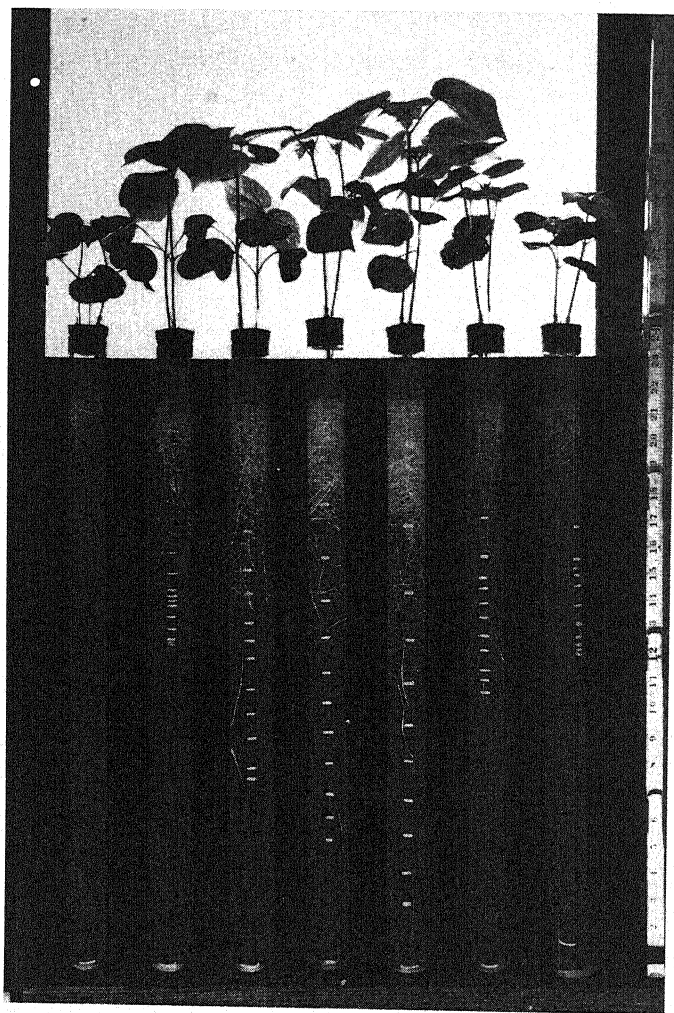


FIG. 4. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 5). Although the gas mixtures were the same as those used in figure 2, the bubbles were not broken up with a sintered glass plate, and the solutions were not kept saturated with oxygen. The "effective" percentages of oxygen were calculated from the determinations of dissolved oxygen. Marks on the tubes represent tap root elongation at 24-hour intervals. The calculated percentages of oxygen and carbon dioxide from left to right were:

O <sub>2</sub>	0	2½	5	10	15	85	95
CO <sub>2</sub>	10	10	10	10	10	10	0

care was exercised in preventing microbial contamination, it was difficult to prevent the roots from being decomposed at the high oxygen concentrations.



If the concentration of oxygen was reduced or if air was substituted, decomposition ceased, and lateral roots developed. Species of *Fusaria* appeared to be dominant. The organisms present were non-parasitic at lower oxygen levels. This interesting observation deserves further study.

The amount of dissolved oxygen in the nutrient solutions receiving the various percentages of oxygen-gas mixtures is given in table I as parts per million. The exact amount of dissolved oxygen varied with temperature. Estimations of dissolved oxygen enabled us to be certain that the rate of gas evolution through the nutrient solutions was sufficient to approximately saturate the solution. It also showed that solutions with no plants growing in them were consistently, though only slightly, higher in dissolved oxygen than solutions containing growing plants. When the rate of gas evolution through the solutions was reduced, the roots absorbed oxygen, and the actual concentration of oxygen in the cultural medium was less than the calculated amount introduced. Figure 4 illustrates the results of a typical experiment of this type. The actual concentrations of oxygen in the solutions was determined by analysis. When 10 per cent. carbon dioxide was used in the mixture, the limiting oxygen factor was well illustrated by reduced root and shoot growth at both low and high concentrations.

#### EFFECT OF FORM OF NITROGEN IN THE NUTRIENT SOLUTION ON OXYGEN REQUIREMENT

The effects of nitrate and ammonium nitrogen on root growth were compared at all oxygen concentrations from zero to 100 per cent. The chief interest was in the effect of nitrate and ammonium nitrogen on cotton root growth at low percentages of oxygen. The results from one such experiment are shown in figures 5 and 6 and summarized in figure 7.

Tap-root elongation was similar with both nitrate and ammonium nitrogen at all concentrations of oxygen. Roots subjected to zero per cent. oxygen remained white and appeared healthy with both nitrate and ammonium nitrogen. Shoot growth seemed greater with nitrate than with ammonium nitrogen, regardless of the oxygen concentration.

Up to 15 per cent. oxygen content tap-root elongation increased, while the weight of the root system was near or at its maximum at two and one-half per cent. oxygen. The lack of correlation between tap-root elongation and increase in weight of the whole root system was caused, at least in part, by the fact that the roots which developed at the lower oxygen concentrations were thick, while those produced at higher percentages of oxygen were slender; thus a given length of root at two and one-half per cent. oxygen weighed more than the same length of root produced at higher oxygen concentrations.

Cotton seedlings growing in solutions containing nitrates accumulated more nitrogen than did those growing in solutions containing ammonia. The extra nitrogen absorbed (in nitrate form) from the solutions containing nitrate over those containing ammonia seemed to accumulate as nitrate



nitrogen within the plants, since the percentages of organic nitrogen in the whole plant was about the same in both the nitrate and ammonia series. The total absorption of nitrogen (in nitrate or ammonium form) increased with oxygen increase up to about two and one-half per cent. The differences in

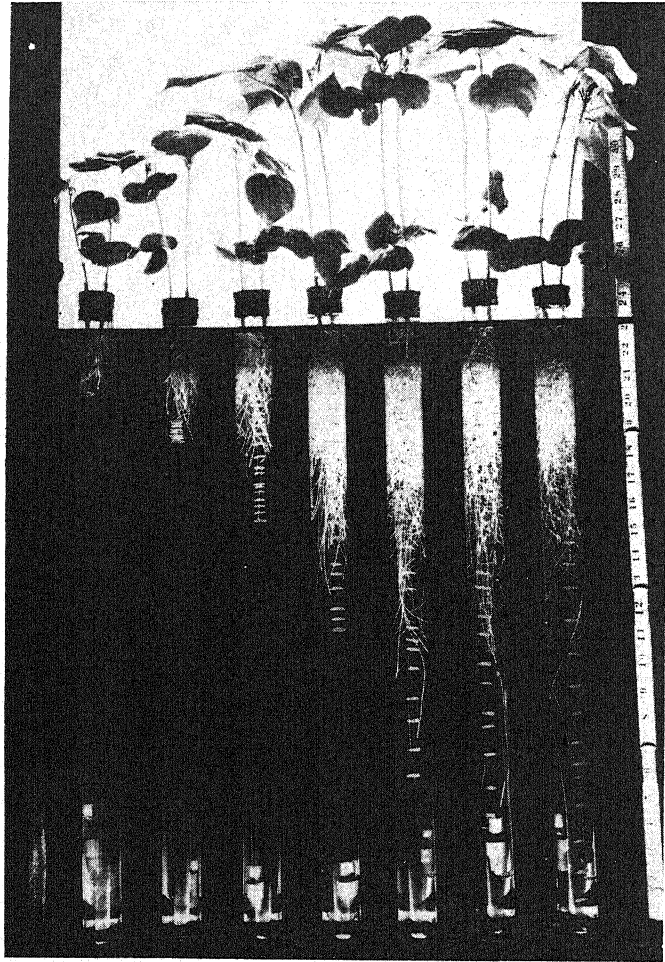


FIG. 5. The effect of nitrate nitrogen on the daily elongation of cotton tap roots and appearance of the shoots at various concentrations of oxygen (Experiment 17). Marks on the tubes represent tap-root elongation at intervals of 24 hours. The gas mixtures from left to right were (percentages):

O <sub>2</sub>	0	1	1½	2½	5	7½	15
CO <sub>2</sub>	0	10	10	10	10	10	10

total uptake of nitrogen were largely caused by differences in plant size, rather than by oxygen concentrations. Nevertheless, the percentage of nitrate nitrogen in the plant increased with oxygen concentration and suggests better absorption of nitrate nitrogen at high than at low oxygen concentrations: The percentage composition is based on an analysis of the whole

plant, and the results may be affected by differences in the relative amounts of roots, stems, and leaves.

In order to study the absorption of nitrate and ammonium nitrogen at different concentrations of oxygen, the nutrient solutions were periodically analyzed. Results with the ammonia series suggested that more ammonium

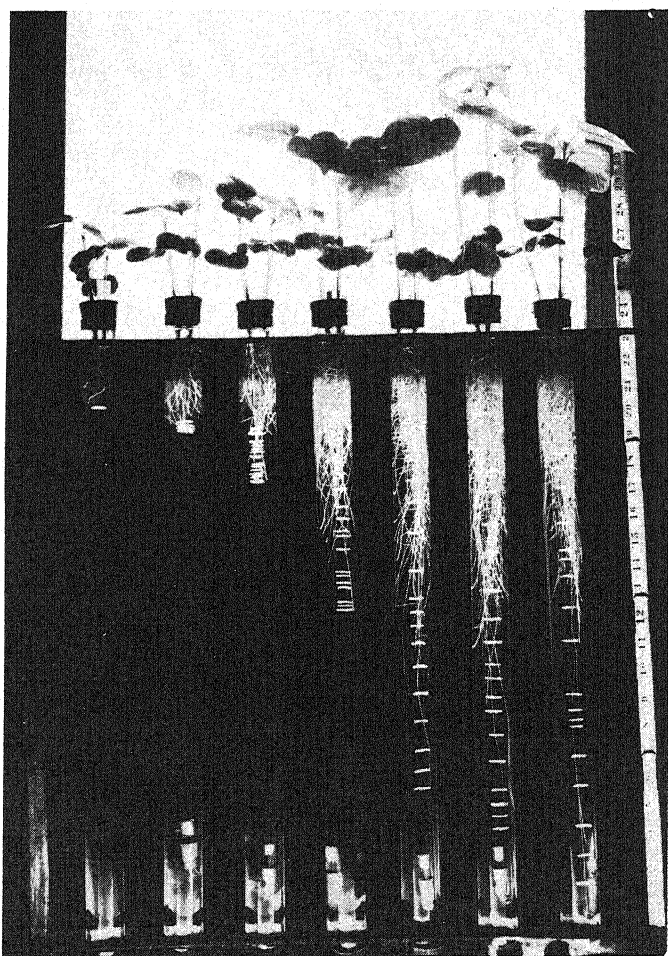


FIG. 6. The effect of ammonium nitrogen on the daily elongation of cotton tap roots and appearance of the shoots at various concentrations of oxygen (Experiment 17). Marks on the tubes represent tap-root elongation at intervals of 24 hours. The gas mixtures from left to right were (percentages):

O <sub>2</sub>	0	1	1½	2½	5	7½	15
CO <sub>2</sub>	0	10	10	10	10	10	10

nitrogen was absorbed at high than at low concentrations of oxygen. The results with the nitrate series indicated the greatest loss of nitrate from nutrient solutions occurred at zero per cent. oxygen. Since a chemical analysis of the plant did not reveal any comparable accumulation of nitrogen

within the plant, it was concluded that some nitrate nitrogen was lost anaerobically from the nutrient solution.

#### EFFECT OF CARBON DIOXIDE ON COTTON ROOT GROWTH

The influence of various concentrations of carbon dioxide on the daily elongation of the cotton tap root is shown in figures 8 and 9. Table II

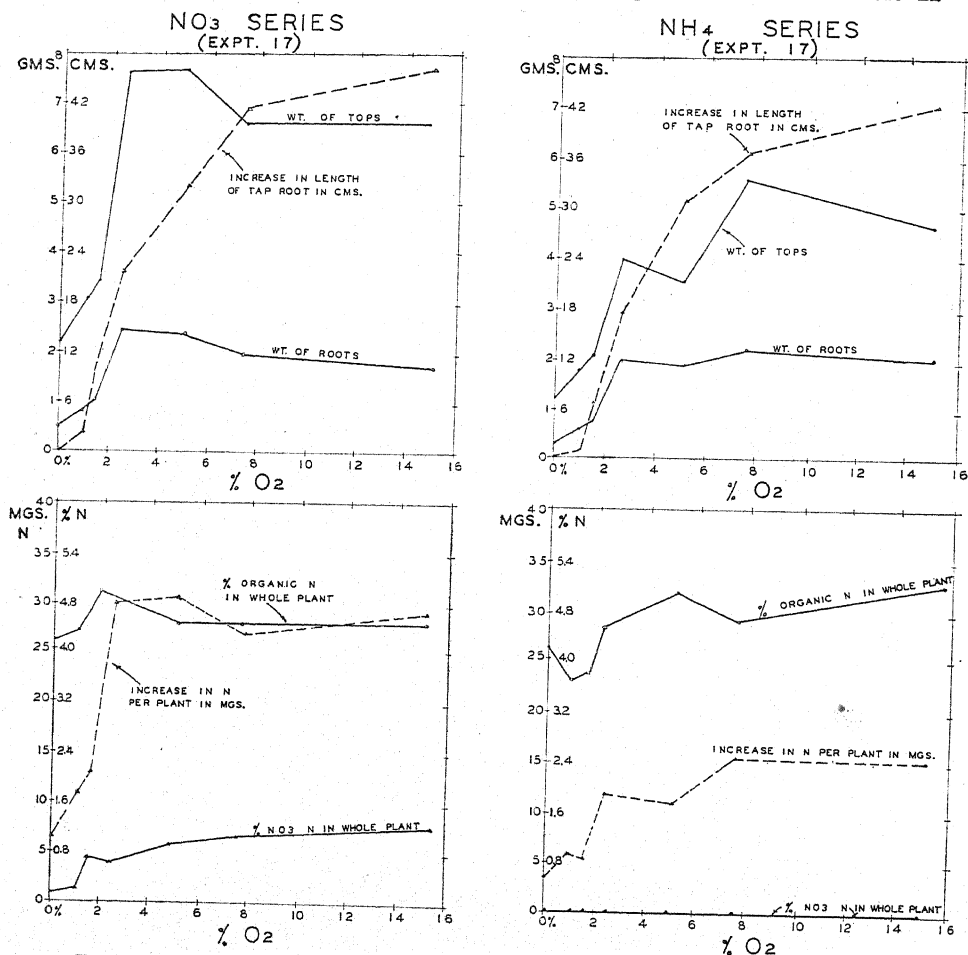


Fig. 7. The influence of various concentrations of oxygen and forms of nitrogen on the total elongation of the tap root, fresh weight of roots and tops, total absorption of nitrogen, and percentage of organic and nitrate nitrogen in seedling cotton plants.

summarizes the results obtained from various experiments in which carbon dioxide was varied. The results of the three experiments shown in figure 8 indicate that a concentration of 60 per cent. carbon dioxide prevented all cotton root growth. Concentrations of carbon dioxide higher than 60 per cent. had the same influence on root growth and appearance of the tops as did 60 per cent. carbon dioxide. The higher concentrations of carbon

dioxide resulted in wilting of the tops, especially when the cotton was in direct sunlight and when the air temperature was above 30° C. Wilting did not occur at the lower concentrations of carbon dioxide, with the exception of occasional wilting at 45 per cent. carbon dioxide.

The pH was controlled by a potassium phosphate buffer and potassium hydroxide. The pH of even the 100 per cent. carbon dioxide solution usually did not shift much below 4.9. Evidently the pH was adequately controlled, even when pure carbon dioxide was bubbled through the nutrient solution. In preliminary tests when the phosphate buffer was not used, pure carbon dioxide produced a pH of 3.6 in the nutrient solution.

Concentrations of carbon dioxide up to 15 per cent. resulted in the production of long, slender roots. Concentrations of 30 to 45 per cent. carbon

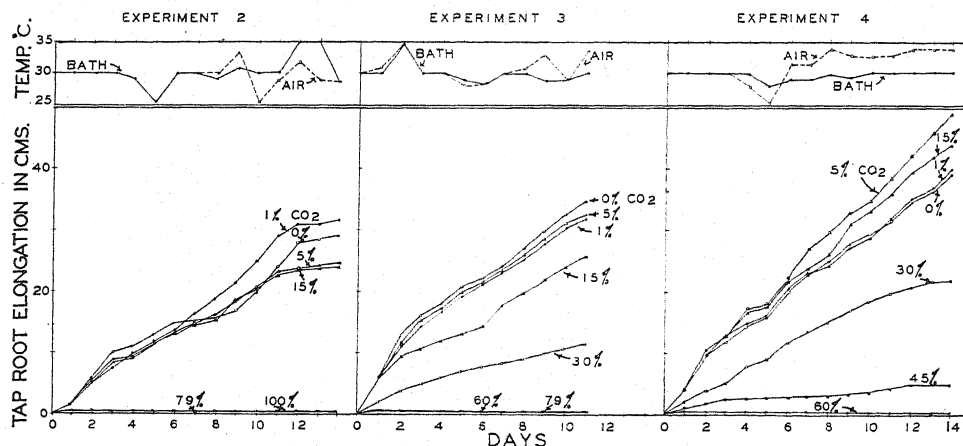


FIG. 8. The effect of various concentrations of carbon dioxide on the daily elongation of cotton tap roots. Oxygen was held constant at 21 per cent., except at 100 per cent. carbon dioxide.

dioxide resulted in the formation of somewhat short, thick roots. Sixty per cent. carbon dioxide, and higher, prevented growth either in length or in thickness of roots.

When 60 per cent. carbon dioxide was replaced by air (21 per cent. oxygen and traces of carbon dioxide) branch roots developed. These results indicated that the pericycle was not destroyed by the high concentrations of carbon dioxide. The tap root was never observed to elongate further, after the introduction of air.

Low concentrations and complete absence of carbon dioxide resulted in no apparent inhibition of root growth. All traces of carbon dioxide were removed by bubbling the gas mixture through gas wash bottles containing potassium hydroxide.

When the temperature of the bath was 35° C. or higher for 24 hours (fig. 8, Experiment 2), root elongation almost ceased at all concentrations, although roots in five to 15 per cent. carbon dioxide were affected most

promptly. The daily elongation of roots growing in the same environment varied for no apparent reason.

### Discussion and conclusions

Cotton tap root elongation was entirely inhibited by gas mixtures containing one-half per cent. of oxygen or less. The roots remained white and apparently healthy under the above conditions for at least two weeks. If

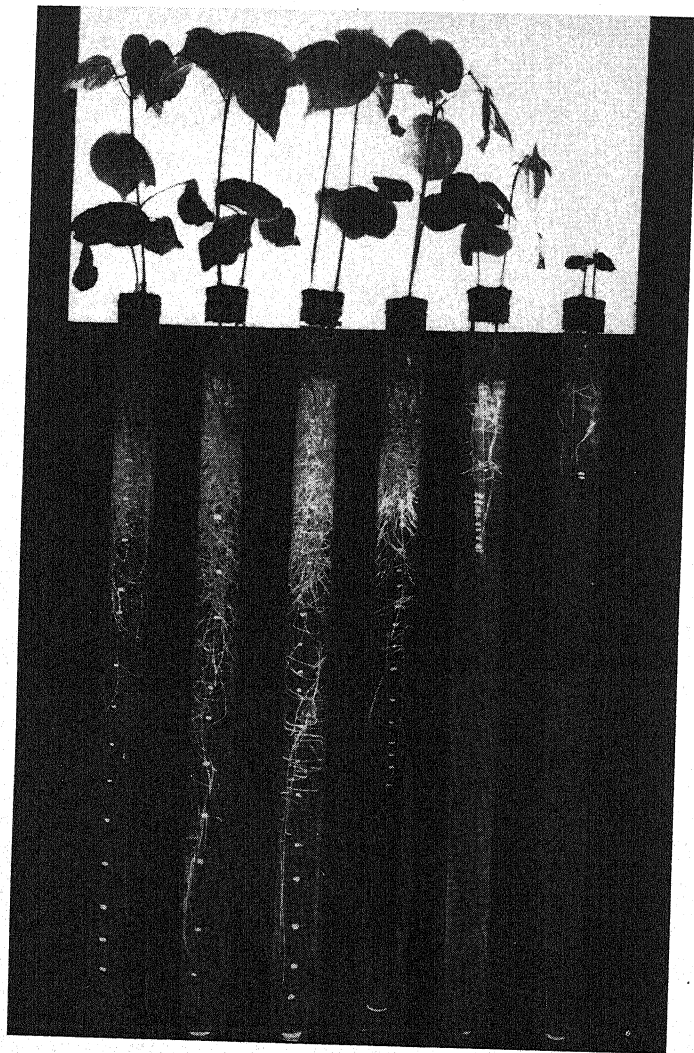


FIG. 9. The effect of various concentrations of carbon dioxide on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 4). Marks on the tubes represent tap-root elongation at 24-hour intervals. The gas mixtures from left to right were (percentages):

CO <sub>2</sub>	0	5	15	30	45	60
O <sub>2</sub>	21	21	21	21	21	21



air containing 21 per cent. oxygen replaced the above gas mixtures, normal branch roots developed, and sometimes the branch roots began to grow again. Evidently, even very drastic reductions in oxygen around cotton roots, with either zero or 10 per cent. carbon dioxide, was not permanently harmful. The roots continued to absorb both nitrate and ammonium nitrogen, although not as well as cotton roots exposed to optimum concentrations of oxygen. The results suggest that the influence of extremely poor aeration of soil on the death of cotton roots is indirect. BALLS (2) observed that cotton roots apparently became asphyxiated when the water table rose. LEONARD (10) observed that when the soil was water-logged and very low

TABLE II

APPEARANCE OF 14-DAY-OLD COTTON SEEDLINGS GROWN IN LIQUID CULTURES SATURATED WITH OXYGEN AND CARBON DIOXIDE AT STATED CONCENTRATIONS.  
RESULTS FROM SEVERAL EXPERIMENTS SUMMARIZED

GASES USED		APPEARANCE OF TOPS	APPEARANCE OF ROOTS
O <sub>2</sub>	CO <sub>2</sub>		
%	%		
21	None	Leaves green, top growth good	Roots thin, very good growth
21	1.0	Do.	Do.
21	5.0	Do.	Do.
21	15.0	Do.	Do.
21	30.0	Do.	Root growth reduced, somewhat thick
21	45.0	Leaves green, reduced top growth	Root growth greatly reduced, thick
21	60.0	Leaves dark green, very little top growth, tending to wilt in sunshine	No root growth. Branch roots developed if transferred to nutrient solution saturated with air
21	79.0	Do.	Do.
None	100.0	Do.	Do.

in oxygen cotton tap roots were short and the tips dead. The influence of poor aeration on cotton roots might easily have been indirect by influencing the susceptibility of the roots to minor parasites.

The studies of VLAMIS and DAVIS (16) indicate that the oxygen requirement of excised roots of tomato, barley, and rice were similar, and yet the three plants reacted differently to anaerobic conditions. The evidence suggests that the reaction of the above plants to anaerobic conditions was related to their capacity to translocate oxygen from the stems to the roots. The capacity of cotton roots to remain alive and function well under anaerobic conditions is also probably related to its capacity to translocate oxygen from the shoots to the roots.

CONWAY (6), using *Cladium mariscus*, found a high oxygen content in intact roots but low oxygen in excised roots under similar conditions. The results suggest that oxygen is translocated to the roots. Differences in the



ability of plants to move oxygen to the roots may in part determine their behavior under anaerobic conditions.

Under anaerobic conditions, short branch roots were produced near the base of the hypocotyl. The tissue was bulky and contained some chlorophyll. Probably sufficient oxygen was present within the tissue of the hypocotyl, either from translocation or from photosynthesis, to enable root initiation.

The minimum concentration of oxygen for cotton tap root elongation was not influenced by the form in which nitrogen was supplied (that is, as nitrate or ammonium nitrogen). The results of VLAMIS and DAVIS (16) likewise indicate that nitrate nitrogen is not superior to ammonium nitrogen for either growth or potassium absorption. SHIVE (15), working with soybeans, found that the greatest absorption of nitrate nitrogen occurred from nutrient solutions containing no oxygen, and absorption of nitrate decreased as oxygen in the nutrient solutions increased. The absorption of ammonia, on the other hand, was the reverse of the absorption of nitrate. The results were explained as indicating that nitrates were supplying oxygen to the roots under anaerobic conditions.

Nitrate nitrogen tended to accumulate in cotton seedlings as the percentage of oxygen in the gas mixtures was increased. In view of the observation that the percentages of organic nitrogen in the whole plants were about the same with different percentages of oxygen, the accumulation of nitrate may signify differences in rates of nitrate absorption. Appreciable accumulation of nitrogen, however, took place even under anaerobic conditions.

PEPKOWITZ and SHIVE (14), working on tomatoes and soybeans, found that the absorption of calcium, phosphorous, and potassium was least at zero parts per million oxygen and rose to an optimum at higher oxygen concentrations; nevertheless, appreciable absorption still occurred, even with the zero oxygen treatment.

VLAMIS and DAVIS (16) found that the accumulation curves for bromide and potassium with different percentages of oxygen were about the same for excised roots of tomato, barley, and rice. A loss of potassium occurred in the absence of oxygen. The accumulation curve for intact roots was quite different from the above. Intact rice roots (shoots attached) in the absence of oxygen accumulated appreciable quantities of bromide; those of barley less; and those of tomatoes almost none.

The lower limit of oxygen necessary for root growth varies somewhat with temperature. According to CANNON and FREE (4) cotton root growth would occur at concentrations of oxygen of slightly less than one per cent., if the temperature was 17° C., but a concentration of over one per cent. would be necessary if the temperature was 30° C. A small amount of cotton root growth occurred at 28° C. with one per cent. oxygen.

In the present investigation the optimum concentration of oxygen for cotton tap root elongation, as well as cotton root growth, was between seven and one-half and 21 per cent. oxygen, although five per cent. oxygen was only slightly inferior. GILBERT and SHIVE (7) reported that the optimum

concentration of oxygen necessary for the production of green matter in soybeans was about six p.p.m.; in tomatoes, 16 p.p.m.; and in oats, four p.p.m.; or approximately equivalent to solutions in equilibrium with 15, 44, and 10 per cent. oxygen, respectively. BOYNTON and COMPTON (3) reported that oxygen concentrations of 15 per cent. or lower resulted in markedly poorer root and top growth of peach, apple, and prune trees growing in nutrient solutions than did 21 per cent. oxygen.

Excessive concentrations of oxygen resulted in reduced cotton root growth. Concentrations of either 90 or 100 per cent. oxygen gave similar results. Besides reducing the daily root growth, high concentrations of oxygen resulted in the roots becoming parasitized by what appeared to be chytrids in some cases and by species of *Fusaria* in others.

Root and shoot growth were reduced at oxygen concentrations above the optimum for root elongation. The leaves, however, appeared normal at the high oxygen concentrations, whether nitrate or ammonium nitrogen was used. GILBERT and SHIVE (7), working with soybeans, found that oxygen values of eight and 16 p.p.m. (equivalent to solutions in equilibrium with 22 and 44 per cent. oxygen) resulted in chlorotic leaves if nitrate nitrogen was present in the nutrient solution, but not if ammonium nitrogen was used. They referred to the chlorotic condition as one of "oxygen toxicity."

Excessive concentrations of carbon dioxide, as well as low concentrations of oxygen, are considered to influence the growth of plant roots in poorly aerated soils. Concentrations of 60 per cent. carbon dioxide, and above, inhibited all cotton root growth even though oxygen was held at an optimum concentration. The pH, likewise, was held constant in these experiments. Evidently, the influence of high concentrations of carbon dioxide on root growth was not caused by a deficiency of oxygen nor by an excessively acid condition of the nutrient solution. If air containing 21 per cent. oxygen replaced the inhibitory concentrations of carbon dioxide, normal branch roots were formed. Evidently, high concentrations of carbon dioxide did not kill the cells of the pericycle. Top growth was very poor when high concentrations of carbon dioxide were used. When the plants were in direct sunlight and the air temperature was 30° C., or higher, the tops wilted.

NOYES (13) passed carbon dioxide through soil in which corn and tomato plants were growing, causing the plants to wilt and growth to stop. CANNON and FREE (4) studied the influence of different concentrations of carbon dioxide on the root growth of a number of plants. They found that high carbon dioxide concentrations inhibited the growth of all plants if the periods of exposure were sufficiently long, but that the sensitivity of different plants to different concentrations of carbon dioxide was not the same. Root growth of *Krameria canescens* ceased if carbon dioxide was 25 per cent. and if the temperature was 30° C.; growth did not cease if the temperature was 20° C. and root growth continued even at 75 per cent. carbon dioxide. Although 25 to 75 per cent. carbon dioxide prevented root growth of *Covillea tridentata*, growth could again occur if the above mixture were replaced by air.

VLAMIS and DAVIS (16) have shown that pure carbon dioxide prevented the accumulation of bromide by both excised and intact roots of barley, tomato, and rice; it also resulted in wilting and cessation of growth. Similar observations on the effect of carbon dioxide on the wilting of plants have been made by other investigators. CHANG and LOOMIS (5) found that carbon dioxide reduced the absorption of water and nutrients by wheat, maize, and rice when compared with aerated cultures of the same plants.

Concentrations of carbon dioxide between zero and 15 per cent. produced no appreciable influence on cotton tap-root elongation. Concentrations of 15 per cent. carbon dioxide occur very rarely in the soil air, as determined by gas analysis. In a previous paper (10) it was pointed out that concentrations of carbon dioxide may be very high adjacent to the surfaces of roots under some conditions, although the occurrence of such concentrations might not be revealed by a gross analysis of the soil air. It is possible that concentrations of even 60 per cent. carbon dioxide are sometimes attained. The concentrations of carbon dioxide at root surfaces under various soil conditions must be determined before the influence of carbon dioxide on root growth in a soil can be evaluated.

Concentrations of carbon dioxide from zero to 100 per cent. were studied in order to determine the influence of all possible concentrations which might possibly influence root growth in the soil. Normal root growth occurred in the absence of carbon dioxide. It does not appear possible, therefore, that minimum concentrations of carbon dioxide can be too low to seriously influence seedling cotton root growth. Further work should be done on the minimum requirements of plant roots for carbon dioxide.

Carbon dioxide is necessary for the growth of many microorganisms. JAHN (9) observed that the growth of the protozoan, *Chilomonas*, was reduced to less than one-fifth in carbon dioxide-free air when compared to ordinary air. LONGSWORTH and MACINNES (11) found that carbon dioxide was necessary for the growth of *Lactobacillus acidophilus*. GLADSTONE, FILDES, and RICHARDSON (8) observed that the passage of carbon-dioxide-free air through cultures of various bacteria stopped growth in some cultures, but not in others. In cultures not inhibited, carbon dioxide was probably formed within the cells themselves. Perhaps the reason cotton root growth was not influenced by the absence of carbon dioxide in the gas mixtures was because all cells of the roots were in contact with adjacent respiring cells; thus no cells were completely subjected to an atmosphere containing zero carbon dioxide, regardless of the composition of the gas mixture around the roots.

Roots in the same environment did not elongate at the same rate from day to day. CANNON and FREE (4) likewise reported similar variations in what appeared to be identical plants. As far as could be ascertained, there was no relationship between root growth, leaf initiation, or leaf area.

#### Summary

A study was made of the oxygen and carbon dioxide requirements of cotton seedling roots in nutrient solutions.

Optimum oxygen concentrations for seedling root elongation of cotton appeared to lie between seven and one-half and 21 per cent. oxygen when carbon dioxide was constant at 10 per cent. The greatest rate of root elongation for any 24-hour period was 67 mm. in 21 per cent. oxygen and 10 per cent. carbon dioxide with culture temperature at 30° C.

The minimum oxygen requirement for cotton root elongation at 28° C. appeared to lie between one-half and one per cent. oxygen. Absence of oxygen around roots did not appear to be very harmful under the conditions studied. When air containing 21 per cent. oxygen replaced nitrogen gas (zero per cent. oxygen), new branch roots were initiated, and some of the old branch roots commenced to grow again.

Ninety or 100 per cent. oxygen resulted in reduced root elongation. Roots produced under such conditions were readily parasitized by chytrids and species of *Fusaria*.

Tap-root elongation at each concentration of oxygen was similar whether nitrate or ammonium nitrogen was used in the culture solution. Vegetative growth appeared improved with the nitrate nitrogen. More nitrate nitrogen than ammonium nitrogen was taken up by the plants.

The optimum concentration of carbon dioxide appeared to range between zero and 15 per cent. when the oxygen concentration was maintained at 21 per cent. The absence of carbon dioxide did not appear to affect root elongation. Concentrations of 60 per cent. carbon dioxide and above prevented all root growth. Root growth was reduced to 30 and 45 per cent. carbon dioxide, and the roots were rather thick.

Shoot growth appeared to be uniform in both height and fresh weight from zero to 30 per cent. carbon dioxide, reduced at 45 per cent. carbon dioxide, and greatly reduced at 60 per cent. carbon dioxide and above. The shoots frequently wilted at 60 per cent. carbon dioxide and above, especially when the plants were in direct sunlight and the temperature was over 30° C.

The writers conclude that the cotton plant is able to withstand anaerobic soil conditions and that this ability is, perhaps, related to the translocation of oxygen from the tops to the roots.

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## ABSORPTION OF WATER THROUGH SUBERIZED ROOTS OF TREES

PAUL J. KRAMER

(WITH ONE FIGURE)

It is generally assumed that absorption of water and solutes by plant roots occurs principally in the unsuberized region back of the root cap where root hairs are usually produced. It seems probable, however, that under some conditions considerable absorption must occur through the suberized regions of roots, especially those of woody plants. For example, root elongation of most trees and shrubs almost or entirely ceases in cold or dry soil, and few or no unsuberized root tips exist at such times. Nevertheless many evergreen species such as pine, privet, holly and citrus lose considerable water by transpiration during such periods. The water lost during periods of little or no root elongation presumably is replaced by absorption through the suberized surfaces of fully differentiated, mature roots. Several investigators have presented indirect evidence that this actually occurs. CRIDER (5) reported absorption of nitrate and phosphate through roots of citrus and vitis after all root tips had been removed and the cut ends sealed. CHAPMAN and PARKER (2) observed absorption of nitrate by orange roots which had turned brown and were at least partially suberized. COLBY (4) stated that apparently apple trees can absorb enough water through suberized roots to prevent wilting but not enough for shoot growth. NIGHTINGALE (7) found that potted apple and peach trees grown at soil temperatures of 45 and 95° F. produced no new roots and some of the old roots even died at 95° F. Nevertheless these trees absorbed water as indicated by the fact that they leafed out and made some shoot growth. Whatever absorption occurred during these experiments must have been through the old suberized roots, or even, in some instances, through dead roots.

In spite of the strong presumptive evidence little direct evidence of water intake through suberized roots has been obtained. In fact the only direct measurements known to this writer are those made by HAYWARD, BLAIR, and SKALING (6) under laboratory conditions on roots of sour orange seedlings. It was decided therefore to make a series of measurements of absorption through suberized roots of trees, especially pines, growing in the open.

### Methods and results

Potometers were made from both glass and rubber tubing. They are shown in figure 1. The glass potometers were slipped over the ends of cut roots and sealed over split stoppers bored to fit snugly around the roots, thus surrounding the root with a water chamber about 2.5 cm. long and 1.0 cm. in diameter. The joints were made watertight with grafting wax or Fisher's Pyseal, the latter being particularly useful in hot weather. Each potometer had two side tubes. To one was attached a small pipette graduated in 0.05



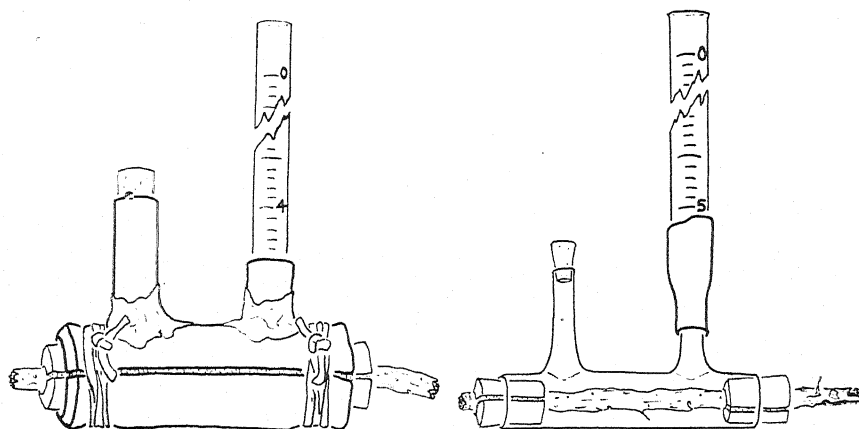


FIG. 1. Drawings of root potometers. Rubber potometer on left, glass potometer on right.

ml. divisions while the other side tube permitted the escape of air during the filling of the potometer, after which it was closed with a stopper. The rubber potometers were made from thick-walled rubber tubing and side tubes were provided by sealing pieces of smaller bore rubber tubing into holes cut near each end. The potometers were split lengthwise so they could be slipped over attached roots without cutting them or uncovering more than 20 or 30 cm. The water chamber was formed by first fastening two split, one-hole stoppers around the roots, then sealing the ends of the potometer to them and tying them tightly with heavy rubber bands. Rubber to rubber joints were sealed with rubber cement and then coated with grafting wax or Pyseal. The diameter of the potometers varied according to the diameter of the roots on which they were placed. Tests made in the laboratory indi-

TABLE I

ABSORPTION OF WATER BY ATTACHED ROOTS OF SHORLEAF PINE  
IN CU. MM. PER SQ. CM. PER HOUR

	TREE NO.	POTOM- ETER NO.	DIAM- ETER OF ROOT	DISTANCE FROM TRUNK	AVER- AGE DAY RATE	No. OBS.	AVER- AGE NIGHT RATE	No. OBS.	DAY/NIGHT
			mm.	cm.	mm. <sup>3</sup>		mm. <sup>3</sup>		
June	1	1	4.8	30	8.35	8	3.52	7	2.3
	2	1	7.0	155	0.99	3	0.55	4	1.8
		2	8.0	248	3.37	8	0.90	7	3.7
	3	1	17.0	36	2.57	6	0.50	7	5.1
		2	4.7	210	1.57	3	1.13	4	1.3
			Average		3.37	.....	1.32	.....	2.8
August	1	1	5.0	25	1.86	2	0.78	2	2.4
	2	1	3.2	20	2.07	2	1.45	2	1.4
	3	1	3.2	25	1.28	2	0.53	2	2.4
			Average		1.73	.....	0.92	.....	2.1

cated that the volume changes caused by change in temperature were not large enough to interfere with measuring absorption.

The roots used varied from 3.0 to 17.0 mm. in diameter and belonged to shortleaf pine (*Pinus echinata* Mill.) trees 8 to 15 feet in height. Care was taken to avoid injury to the surface of the roots, and potometers were located on segments free of side branches and visible injuries. Results of several days observations are summarized in table I. Measurable volumes of water were absorbed during both day and night by roots of all diameters. Simultaneous observations were made with potometers attached at various distances from the trunk, but no correlation was apparent between absorption and distance of potometer from the tree. When measurements were made in June the soil was very dry, the temperature was high, and conditions were favorable for high transpiration; but the August measurements were made a few days after the soil had been thoroughly wetted by rains, and atmospheric conditions were less favorable for transpiration. Both series were made on the same trees. As would be expected, more water was absorbed from the potometers per unit of root surface when the soil was dry than when

TABLE II

AVERAGE DAY RATE OF ABSORPTION OF SHORLEAF PINE ROOTS  
IN CU. MM. PER SQ. CM. PER HOUR

From water .....	3.37	Average of 28 determinations
From soil .....	2.63	Average of 6 determinations

it was moist. The results are summarized in table I. Measurement of water intake at two-hour intervals during the day indicates that the rate of absorption is probably closely related to the rate of transpiration, but lags behind it somewhat so that considerable absorption occurs at night.

Attempts were also made to measure the rate of absorption from moist soil. A layer of soil moistened to field capacity was held in close contact with roots by means of pieces of rubber tubing which enclosed soil and root. The rate of absorption from soil initially wetted to field capacity averaged about as high as absorption from water (table II). These experiments lasted 12 hours, from 6 A.M. to 6 P.M. Presumably the rate would decrease markedly after the available water was removed from the soil in immediate contact with the roots.

A comparison of the rates of water movement through pine and hardwood roots was also made. It was decided that this could not be done on roots attached to trees because it would be impossible to determine whether differences in rate of water intake resulted from differences in permeability or from differences in rate of absorption of the trees to which the roots were attached. Comparisons were therefore made in the laboratory by sealing one end of root segments and attaching the other end to a vacuum line and immersing the root segments in water. Thus the permeability to water could be compared under similar conditions. The species used were dogwood and

shortleaf pine. The data obtained are summarized in table III and indicate that dogwood roots are considerably more permeable to water than pine roots.

TABLE III

RELATIVE PERMEABILITY OF PINE AND DOGWOOD ROOTS. ROOT SEGMENTS WERE ATTACHED TO A VACUUM LINE AND SUBJECTED TO 30 CM. PRESSURE. RATES IN CU. MM. PER SQ. CM. PER HOUR

SPECIES	NUMBER OF DETERMINATIONS	AVERAGE RATE
		<i>mm.<sup>3</sup></i>
Shortleaf pine .....	19	9.0
Dogwood .....	5	15.6
Yellow poplar .....	4	101.4

### Discussion

These results indicate that appreciable quantities of water can be absorbed through suberized roots, even through roots one or two cm. in diameter which are covered with a relatively thick layer of bark. No information is given, however, as to the manner in which water passes through the bark. HAYWARD, BLAIR, and SKALING (6) stated that water entered orange roots chiefly through the numerous lenticels. Studies of the path of water movement in pine, yellow poplar and sweet gum have been made by a colleague and will be reported in another paper (1).

It is not intended to depreciate in any way the importance of unsuberized root tips and of root elongation in the absorption of water. There are times, however, when the soil is too cold or dry for root elongation to occur, and few or no unsuberized root tips exist. At such times absorption through suberized roots may be of major importance. The number of small suberized roots in the soil under a forest stand is large enough to possess considerable surface and absorb an appreciable amount of water (3). Since the soil is usually moist during the winter when root growth is slowest, conditions are such that most or all of the water required by evergreen trees probably can be absorbed through the mature, suberized roots. Even in the summer some water is doubtless absorbed through them, particularly when a rain follows a drought during which root elongation has ceased, and the roots have become suberized to their tips. Several days would be required following a rain for root growth to be resumed, but absorption through the older portions of the roots doubtless begins immediately.

Absorption through older roots of herbaceous species doubtless also occurs under certain conditions. In a recent study (9), for example, it was found that the seminal roots of a number of perennial grasses remained alive for 3.5 to 4 months. Although the cortex had sloughed off the older portions of these roots, it seems probable that even the decorticated portions function as absorbing surfaces, at least in moist soil. It has been reported (8) that certain grasses have perennial roots; that is, most of the roots continue to

function the second season. Considerable absorption must occur through such roots. It seems probable, therefore, that absorption through older roots in which secondary growth has occurred is a fairly common occurrence, especially at times when few roots are elongating.

### Summary

The rate of water absorption through suberized roots of shortleaf pine was determined by attaching potometers to roots of small trees growing in the open. The roots used varied in diameter from 3.2 to 17.0 mm. and were covered with thick layers of cork. Measurable absorption occurred through all roots studied. Absorption of water from moist soil by suberized roots also occurred.

Comparison of the rates of water movement through segments of pine and dogwood roots indicate that dogwood roots are much more permeable to water than pine roots.

Absorption of water through suberized roots is probably of vital importance in evergreen trees during the winter when root growth is much reduced or ceases and the roots become suberized to their tips. It is probably also of some importance in the summer, particularly immediately following a drought which has stopped root elongation.

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## MINERAL REQUIREMENTS OF *LEMNA MINOR*

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### Introduction

The use of *Lemna minor* Link as a test organism for the micronutrient requirements of the higher plants was undertaken by the writer as a check on similar data with the fungus *Aspergillus niger* v. Tiegh. It was possible to demonstrate that the macro- and micronutrient needs of both were similar, except that *Lemna* also required calcium and boron (1). The mineral and acidity requirements of *Lemna* were identical with those of other higher green plants.

In *Lemna* cultures with *fixed, minimal quantities of salts*, the ratio of yield to total macronutrient salts was 0.9, a value termed "efficiency of salt utilization" by the writer. This corresponded to a total of 1,113 mg. of macronutrients per gram of yield, each salt of the nutrient solution having been adjusted to be at the experimental minimum for maximum yield. Addition of 0.5 per cent. sucrose, nevertheless, increased the ratio to 2.3 though the quantity of salts remained unaltered. The macronutrient salt requirement was thereby decreased to 434 mg. per gram of yield. *Aspergillus*, however, reaches an efficiency of 9.6, which is equivalent to 106 mg. salts per gram. The lower efficiency of *Lemna* may be intrinsic but might be only a reflection of secondary requirements, such as buffering of the nutrient solution and insufficient energy (low photosynthesis) for efficient absorption.

An effort has been made to obtain an improvement in the results previously reported. Numerical data on the effects of copper are also included.

### Methods

*Lemna minor* Link was grown on 50 ml. of nutrient solution in 200-ml. Erlenmeyer flasks under aseptic conditions. Illumination was continuous at 500 foot-candles supplied by 3,500° C. white fluorescent lamps. The temperature was  $25^{\circ} \pm C$ . Photosynthesis is the limiting factor for growth under these conditions (1). Only quartzware was used. Nutrient solutions were prepared with water twice redistilled in quartz and standard reagent chemicals having a purity sufficient for chemical analyses. Micronutrients, however, were almost spectroscopically pure chlorides. The sucrose contained 0.0011 per cent. ash. Each experiment was started with a single plant (having two fronds) per flask. The roots usually varied from 2 to 5 mm. in length. Duration of growth was 13, 14, or 27 days with continuous illumination. No change was made in the solutions of the individual flasks for the duration of the experiments. The plants were dried at  $103-5^{\circ} C$ . for 3 to 4 hours before weighing.

## Results

## EFFECTS OF MICRONUTRIENT DEFICIENCIES

The effects of micronutrient deficiencies on yields are shown in table I. Selected reagent chemicals were used in various combinations. The solutions used were adjusted to minimum salt content for maximum yield. Sharp diminutions in growth were readily obtained on omission of iron, manganese, molybdenum, and boron. Results with zinc, copper, and gallium were less clear cut.

TABLE I

EFFECTS OF MICRONUTRIENT DEFICIENCIES ON GROWTH OF LEMNA WITH  
SELECTED REAGENT CHEMICALS

ELEMENT OMITTED	SELECTED REAGENT CHEMICALS*											
	13 DAYS pH = 4.17			13 DAYS pH = 4.06			13 DAYS pH = 4.10			27 DAYS pH = 4.30		
	YIELD	RELATIVE YIELD	APPEARANCE†	YIELD	RELATIVE YIELD	APPEARANCE†	YIELD	RELATIVE YIELD	APPEARANCE†	YIELD	RELATIVE YIELD	APPEARANCE†
	mg.	%		mg.	%		mg.	%		mg.	%	
None	13.2	100.0	4,M	15.6	100.0	4,M	14.0	100.0	4,M	60.1	100.0	4,M
Fe	2.5	18.9	1,S	3.3	21.2	2,S	2.1	15.0	1,T	3.9	6.5	1,S
Zn	12.1	91.7	4,M	13.3	85.3	4,M	11.7	83.6	4,M	44.7	74.4	2,M
Cu	10.6	80.3	4,M	13.9	89.1	4,M	7.8	55.7	4,M	34.8	57.9	4,M
Mn	3.1	23.5	2,T	2.9	18.6	2,S	1.7	12.1	1,T	2.5	4.2	1,T
Mo	4.3	32.6	2,M	3.2	20.5	2,S	3.1	22.1	2,S	40.5	67.4	4,M
Ga	12.2	92.4	4,M	15.4	98.7	4,M	8.9	63.7	4,M	55.7	92.7	3,M
B	5.4	40.9	5,T	9.2	59.0	5,S	5.2	37.1	5,S	43.4	72.2	5,S

\* First 3 experiments: Water, 1 liter;  $\text{KNO}_3$ , 50;  $\text{KH}_2\text{PO}_4$ , 50;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 30; Fe, 0.20; Zn, 0.05; Cu, 0.01; Mn, 0.05; Mo, 0.02; Ga, 0.02; B, 0.02; Ca, 4.0 mg. Last experiment: Water, 1 liter;  $\text{KNO}_3$ , 300; KCl, 50;  $\text{KH}_2\text{PO}_4$ , 70;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 45; Fe, 0.40; Zn, 0.10; Cu, 0.02; Mn, 0.20; Mo, 0.06; Ga, 0.04; B, 0.06; Ca, 8.0 mg.

† Color is rated from 0 to 5 (normal green); and size of fronds designated as L=large, M=medium, S=small, T=tiny.

Symptoms of micronutrient deficiency followed a parallel course. Normal plants had large green fronds with flat, rounded apices and long, light green roots. With insufficient iron, the plants remained adherent; the fronds were tiny with distorted apices and displayed a diffuse chlorosis, usually progressing from apex to base of frond. Roots were short, light green, and sometimes distorted. Deficiencies in zinc, gallium, and particularly copper were sporadically accompanied by a general diffuse chlorosis of fronds very similar to that shown in absence of iron.

In the absence of manganese the plants remained adherent and had very small fronds with sharply defined white margins and elongated blotches giving a green "trident effect" on all but the base, and occasional red spots. Roots were short, twisted, and dark green. Omission of molybdenum also resulted in adherent plants with small fronds, many of which had acute up-



turned apices. Chlorosis was diffuse with white blotches merging into green and often accompanied by narrow marginal streaks of dark green. The roots were long, twisted, and very dark green. Non-addition of boron led to plants markedly adherent, having tiny and very dark green fronds with occasional acute apices and red spots. Roots were very short, dark green, and kinky.

#### NUTRIENT SOLUTION PURIFICATION

Table II contains experiments illustrating the effects of nutrient solution purification (1) on micronutrient deficiencies. Improved results were obtained with iron, zinc, and copper as compared to unpurified solutions composed of selected reagents. Results with gallium were slightly better when calcium phosphate was used instead of calcium carbonate. Details of this process will be found in the publication cited above. The removal to a greater or lesser extent of residual traces of iron, zinc, copper, etc., impurities from the reagent chemicals is accomplished by neutralizing the complete nutrient solution with an excess of  $\text{CaCO}_3$ ,  $\text{CaHPO}_4$ ,  $\text{CaO}$ , or the corresponding magnesium salts, in the presence of excess phosphate. The sediment formed is an important factor in the treatment, since it serves also as an adsorbent for micronutrient impurities. The clear solution is used and the sludge rejected.

These solutions contained: water, 1 liter;  $\text{KNO}_3$ , 400;  $\text{K}_2\text{HPO}_4$ , 440;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 350;  $\text{CaCO}_3$ , 250; and  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ , 750 mg. After heating in a steamer for 20 minutes, the solutions were filtered cold, and 2.0–2.5 ml. of N/1 hydrochloric acid added. Micronutrients per liter then added were: Fe, 0.40; Zn, 0.04; Cu, 0.01; Mn, 0.075; Mo, 0.02; Ga, 0.02; B, 0.04 mg.

#### EFFICIENCY OF SALT UTILIZATION

Previous work (1) had indicated that addition of sucrose to a *Lemna* culture resulted in an increase in growth even though each nutrient component was presumably at the experimental minimum for maximum normal growth. The optimum macronutrient mixture of 32.5 mg. salts that was essential for a yield of 29.2 mg. without sucrose sufficed for the production of 74.9 mg. on addition of 0.5 per cent. sucrose. This is equivalent to a decrease in salt requirements for growth with an increase in level of carbon nutrition and is, therefore, referred to as an increase in "efficiency of salt utilization." The corresponding ratios 29.2/32.5, or 0.9; and 74.9/32.5, or 2.3 are reciprocals of salts per unit of yield.

Salt utilization ratios with *Lemna* have been given further attention in table III. The data summarized should suffice to demonstrate each of several points. Normal growth is dependent on quantity ratios of nutrients irrespective to total quantity. Available sugar is closely correlated with total quantity of salts required for growth. Increased salt utilization efficiency with increased carbon nutrition takes place with chlorotic as well as normal plants.

An intensity of 500 foot-candles is known to be sub-optimal for growth

TABLE II  
EFFECT OF NUTRIENT SOLUTION PURIFICATION ON RESPONSES OF LEMNA TO MICRONUTRIENT DEFICIENCIES (14 DAYS)

ELEMENT OMITTED	CaCO <sub>3</sub> PURIFICATION						CaHPO <sub>4</sub> · 2H <sub>2</sub> O PURIFICATION					
	SUCROSE 0.1% pH = 4.01			NO SUCROSE pH = 4.05			SUCROSE 0.1% pH = 4.32			NO SUCROSE pH = 3.97		
	YIELD	RELATIVE YIELD	APPEARANCE*	YIELD	RELATIVE YIELD	APPEARANCE*	YIELD	RELATIVE YIELD	APPEARANCE*	YIELD	RELATIVE YIELD	APPEARANCE*
None	mg.	%		mg.	%		mg.	%		mg.	%	
Fe	44.7	100.0	4, M	15.8	35.3	4, L	29.7	66.2	4, L	22.0	49.2	4, L
Zn	1.8	4.0	1, S	0.6	33.3	1, T	1.5	83.3	1, S	1.2	66.7	2, S
Cu	21.2	47.4	4, L	7.6	35.8	4, M	22.1	104.2	4, L	11.4	53.8	4, L
Mn	16.0	35.8	4, L	10.6	66.1	4, M	24.3	151.9	3, L	12.4	77.5	4, L
Mo	3.3	7.4	2, S	1.0	30.3	1, T	5.3	160.6	2, S	1.3	39.4	1, S
Ga	27.2	60.9	4, L	6.1	22.4	4, M	10.1	36.4	4, L	7.5	27.2	4, L
B	42.1	94.2	3, L	15.2	36.1	3, M	25.9	61.5	4, L	16.6	39.4	4, L
	16.5	36.9	5, S	12.1	73.3	4, M	27.2	161.8	4, L	12.0	72.7	4, L

\* See footnote, table I.

of *Lemna* (1) which requires 1500 foot-candles. Air enriched with  $\text{CO}_2$  is ineffective in increasing yields except in experiments of over 2 weeks duration when the leaf surface increases to the point where air diffusion through the cotton plugs of the flasks becomes inadequate. Light is the limiting factor for growth during the early stages of the experiment and carbon dioxide during the later stages. Sucrose was effective at all stages in increasing growth, whereas manipulation of macro- and micronutrients was ineffective, as were also addition of trace elements or vitamins. The action of

TABLE III

EFFICIENCIES OF SALT UTILIZATION (YIELD/TOTAL AVAILABLE MACRONUTRIENT SALTS)  
OF *Lemna minor* GROWN FOR 27 DAYS AT 25° C. WITH 500 FOOT-  
CANDLES OF FLUORESCENT LIGHT

NUM- BER	MILLIGRAMS PER CULTURE OF 50 ML.				MILLI- GRAMS YIELD PER CULTURE	SUPPLEMENT	YIELD DIVIDED BY TOTAL AVAILABLE SALTS
	$\text{KNO}_3$	$\text{KH}_2\text{PO}_4$	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	TOTAL SALT			
	mg.	mg.	mg.	mg.	mg.		
1*	15.0†	3.5	2.25	23.25	61.0	.....	2.6
2	17.5	10.0	5.0	32.5	63.2	.....	1.9
3	7.5‡	3.25	2.0	15.75	47.6	.....	3.0
4	7.5‡	3.25	2.0	15.75	191.0	0.5% sucrose	12.1
5	4.5	3.0	2.0	9.5	47.8	.....	5.0
6	4.5	3.0	2.0	9.5	60.8	$\text{CO}_2$	7.2
7	4.5	3.0	2.0	9.5	85.6	<i>A. niger</i> vapors	9.0
8	4.5	3.0	2.0	9.5	106.4	<i>A. niger</i> vapors	11.2
9	6.0‡	3.25	2.0	14.25	40.6	.....	2.9
10	6.0‡	3.25	2.0	14.25	83.5	0.1% sucrose	5.9
11	6.0‡	3.25	2.0	14.25	134.1	0.2% sucrose	9.4
12	6.0‡	3.25	2.0	14.25	173.6	0.3% sucrose	12.2
13	6.0‡	3.25	2.0	14.25	198.6	0.4% sucrose	13.9
14	6.0‡	3.25	2.0	14.25	222.7	0.5% sucrose	15.6
15	6.0‡	3.25	2.0	14.25	232.5	0.6% sucrose	16.3

\* Experimentally determined to contain a minimal quantity of each salt for maximum normal growth under the specific environmental conditions employed. All other cultures were chlorotic. Micronutrient concentrations as in table I, last experiment.

† Plus 2.5 mg. KCl or  $\text{KNO}_3$  per culture.

‡ Plus 3.0 mg.  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  per culture.

sucrose is, therefore, assumed to depend on an increase in the level of carbon nutrition of *Lemna*.

The minimal quantities of macronutrient salts required for maximum normal growth are shown in experiment 1. The yield was 61 mg. per culture and required 23.25 mg. of macronutrients; i.e., a ratio of 2.6. Neither an increase (#2) nor a decrease (#3) in individual or total components of the solution increased yield but did cause chlorosis. Addition of sucrose (#4) brought about a 3-fold increase in yield and a 4-fold increase in salt utilization ratio without eliminating chlorosis. Experiments 5 to 8 illustrate similar increases in salt utilization ratios with  $\text{CO}_2$  and with vapors from cultures of *Aspergillus niger* v. Tiegh. The remaining experiments show the effect of increasing quantities of sucrose on yield and utilization ratios

despite the fact that nutrition was abnormal and the plants chlorotic. Sucrose caused a 6-fold increase in yield despite chlorosis.

These data would indicate, therefore, that nutrient salts required for growth of *Lemna* are, for reasons unknown, far in excess of the plant's actual requirements for formation of tissue. Usually 38.5 per cent. of salts (1/2.6) must be provided on the basis of anticipated yield. With sucrose, 6.1 per cent. of salts (1/16.3) sufficed. This value approaches that mentioned by PFEFFER in his treatise of plant physiology, where he points out that a minimal salt solution for the oat plant should require only about 2.5 per cent. salts per unit of yield. This computation was based on the experimental optimum for each element.

Though it has been possible to approach this value with *Lemna*, the problem remains of doing so without causing chlorosis. The causes of these chloroses were sought in experiments with macronutrient salts and trace elements, but unsuccessfully. The further addition of  $\text{KNO}_3$  [also  $\text{Ca}(\text{NO}_3)_2$  or  $\text{KCl}$ ] prevented chlorosis under these conditions but only in association with marked decreases in efficiency of salt utilization.

### Discussion

Improved results with micronutrient deficiencies were obtained through selection of nutrient salts and their use in minimum quantities. Decreases in growth due to their omission from the nutrient solution were accompanied as a rule by specific symptoms in the case of iron, manganese, molybdenum, and boron. Symptoms of zinc, copper, and gallium deficiency were sporadic and consisted in uniform chloroses of older fronds.

The salt utilization studies with *Lemna* have been based on the use of limited quantities. That is to say, the quantities initially supplied were used at the experimental minima for maximum normal growth and were not replenished to compensate for depletion. Quantity ratios and total quantity were found to be important factors for growth under these conditions (2). An indefinite and variable proportion of the total salts supplied did not appear to function primarily in the elaboration of tissue by the plant. The apparent increase in effectiveness of the salt supply in maintaining growth on addition of sugar would indicate this to be so and also that maximum efficiency of salt utilization would be reached at the optimum carbon nutrition level of the plant. Hindrance of salt absorption because of low carbon nutrition level and buffer requirements may be factors in higher mineral needs. The carbon nutrition level, therefore, appears to be the determining factor for total salt requirements in the case of *Lemna* as well as *Aspergillus* (2).

### Summary

*Lemna minor* Link was grown in aseptic culture for 13, 14, or 27 days at 25° C. with continuous light (500 foot-candles, fluorescent lamps) using fixed, limited quantities of nutrients. The effects of deficiencies in micronutrients are described. Efficiency in salt utilization was found to be depen-

dent on the carbon nutrition level of the plant and reached a maximum value of 16.3 (yield/salt) or the equivalent of 6.1 per cent. salts for each unit of yield. Quantity and quantity ratios of nutrient salts were important factors in growth.

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## INFLUENCE OF EXTERNAL AND INTERNAL FACTORS ON GROWTH HORMONE IN GREEN PLANTS<sup>1</sup>

FELIX G. GUSTAFSON

Over a period of several years the writer has made numerous determinations of growth hormone content in plants, as influenced by external and internal factors. The external factors studied were mineral nutrition, light, temperature, and parasites; internal factors studied were chlorophyll, dwarfness, age of the plant, and position on the plant of an organ. Other studies of this sort have been published and will be referred to under the appropriate section.

### Materials and methods

Hormone tests were made by the Avena method (17) on extracts from the plant. Several methods have been employed in making the extracts, but differences in extraction method are not important because comparisons are made only within an experiment or between experiments conducted on the same day in an identical manner, a procedure obviating the necessity of making calculations in terms of indoleacetic acid equivalent or any other equivalent. In most of these experiments direct comparisons are made of Avena curvatures obtained with different materials when the procedure of extraction and auxin determination were identical and carried out at the same time with the same lot of Avena plants. In most of these experiments the same amount of plant material was used, and in the final analysis the same dilution was used; i.e., the same amount of agar was added to comparable amounts of extract. This procedure eliminates many objections that have been raised against some of the methods used in stating auxin content in plants.

Several different plant species have been used, and these will be mentioned as they come up; the main experimentation has been made with the John Baer tomato, though corn has also played an important part in the investigation.

### Results

#### INFLUENCE OF SOIL FERTILITY ON AUXIN CONTENT OF TOMATO PLANTS

In a study of growth hormones, a comparison between growth and hormone content is very important; as one means of regulating growth is by varying the mineral nutrition, this method has been used. AVERY, BURKHOLDER, and CREIGHTON (1, 2) found that vigorously growing sunflower and Nicotiana plants had a high concentration of auxin whereas poorly growing plants had less hormone; and that field-grown Nicotiana plants showing

<sup>1</sup> Paper from the Department of Botany and the Botanic Garden of the University of Michigan, no. 746. This investigation has been aided financially by the HORACE H. RACKHAM Trust Fund of the University of Michigan.

deficiency symptoms had as much hormone as controls. SKOOG (12), on the other hand, found that zinc-deficient tomato plants had much less hormone than plants supplied with zinc. WENT (18) found a slight reduction of hormone with low nutrition of tomato.

In the present experiments, John Baer tomatoes were grown in soil under conditions similar to those used in commercial greenhouses except that the plants were grown in large pots instead of in the ground. Three soils were used: soil I, composed of ordinary field soil without any addition of fertilizers; soil II, an artificial compost made from turf, manure, dried blood, German peat, and  $(\text{NH}_4)_2\text{SO}_4$ , and permitted to decompose for a period of three years; and soil III, which was soil II to which had been added one  $3\frac{1}{2}$ -inch pot of bone meal and one of Vigoro per bushel of soil. During the early part of the experiment the plants were grown in 6-inch pots but as they became larger they were transferred to 9-inch pots. At the time of repotting, soils II and III were refertilized.

TABLE I

INFLUENCE OF SOIL FERTILITY ON THE AUXIN CONCENTRATION IN TOMATO PLANTS. DEGREE OF CURVATURE OF THE AVENA COLEOPTILES DENOTES HORMONE CONTENT. FOR FURTHER INFORMATION ON THE METHOD OF INDICATING HORMONE CONCENTRATION SEE THE TEXT

EXP.	DATE	CURVATURE OF AVENA COLEOPTILES		
		SOIL I	SOIL II	SOIL III
211	11/23/42	13.8	14.9	15.1
212	12/ 2/42	4.8	6.8	8.0
220	1/11/43	3.9	9.4	6.9
227	3/31/43	1.6	4.9	5.1

Four determinations of auxin were made; for each determination two or three leaves from near the tip of the plants were used. On November 23, when the first sampling was made, the plants in soil I were about 4 feet tall, thin, and spindling with the lower leaves yellow and with one or two fruits per plant. The plants in soils II and III were more vigorous with dark green leaves but had no fruits. On December 2, when a second collection of material was made, the plants were essentially in the same condition as in November. After gathering the material necessary for the test, the plants were pruned and repotted in 9-inch pots. The third collection was made on January 11, and at this time the plants in soil I showed signs of extreme starvation; they had no flowers or flower buds, the leaves were small, and the veins were purple (lack of phosphorus); the other plants had flowers, flower buds, and fruits, and the leaves were large and green. The last collection was made on March 31; at that time the plants in soil I had very small leaves, veins and stems showed deep purple color, and the stems were thin and not very tall. The other plants were vigorous, with many flowers and large green leaves, the plants in soil III being the most vigorous.

The relative hormone content is denoted by the degree of curvature of

*Avena coleoptiles*. In a particular experiment the amount of plant material used was the same from each soil, and the amount of agar added to the extract was the same, so a direct comparison can be made between the plant lots.

It is evident that poor soil produces poor plants and that poor plants have a much lower hormone content than vigorous plants. It is equally evident that increasing the soil fertility beyond a certain point does not increase the vigor of the plants or their hormone content. In the early part of the experiment the plants grown in soil I, though smaller than the others, were still quite vigorous, and at that time their hormone content was very near to that of the others; but as the nutrient of the soil became depleted, the hormone content decreased greatly. At the beginning of the experiment, soils II and III were too rich for the setting of fruits; but later, as the soils became depleted, the plants fruited abundantly. The repotting of the plants, and especially the addition of fertilizers to soils II and III at that time, increased the hormone content of the plants slightly. The experiments further bring out the fact that phosphorus starvation is associated with low hormone content; but as the plants in soil I were lacking in vigor due to lack of other elements also, not too much emphasis should be placed on the lack of phosphorus.

#### INFLUENCE OF LIGHT UPON THE AUXIN CONTENT OF GREEN PLANTS

This experimentation was initiated to discover whether in hormone studies time of day at which plant material is collected has any significance; but later the experiments were more concerned with the influence of light upon the hormone concentration.

The preliminary experiments in which material was collected in the morning and late afternoon, from plants grown either in the field or in the greenhouse, were not very satisfactory. In a general way it can be stated that, when there was any difference between the two collections, the afternoon collection had a lower hormone content than the morning collection if the light intensity was great, as it would be on a sunny day in the summer; but if, on the other hand, the day was cloudy, or if the time was winter, the evening collection was likely to have more hormone. In all of these experiments, tomato plants about 3 to 4 feet tall were used, grown either in the field or greenhouse. While not very satisfactory, these experiments nevertheless showed the desirability of collecting the material at the same time each day and preferably around noon. This schedule has been followed quite consistently in this laboratory for some years.

The experiments just mentioned led to others in which plants of different sizes grown in the greenhouse were kept in the dark for various lengths of time. Some investigators (3) had shown that in *Nicotiana* there was a rapid disappearance of the hormone when the plants were kept in the dark room, while others (5) had shown that seedlings grown in the dark and then exposed to light suffered a loss of hormone as a result of the light stimulation

and still others (18) have shown that plants in dark have more hormone than plants in the light. These results seem, at least on the surface, contradictory.

A group of similar plants were selected and half of them placed in a darkroom while the others were left in the greenhouse (table II). In the darkroom, which was connected to the greenhouse by a ventilator, a fan was continuously sucking in air which left the room by another opening. Thus, except for the middle of hot days, the temperature and relative humidity were the same in the two rooms. As will be noticed, the time of these experiments extended over a period of one year, a period during which various light and temperature conditions were encountered. Except for three experiments, the John Baer tomato was used as the experimental plant.

TABLE II

GROWTH HORMONE CONTENT IN PLANTS SUBJECTED TO NORMAL LIGHT CONDITIONS AND TO CONTINUOUS DARKNESS FOR DIFFERENT LENGTHS OF TIME

EXP.	DATE	CURVATURE OF AVENA PRODUCED BY PLANTS FROM		HOURS IN DARK	KIND AND SIZE OF PLANTS
		GREEN- HOUSE	DARK- ROOM		
263	1/24/44	6.3	7.1	26	Tomato, 2 ft. tall
			7.3	50	
264	1/31/44	11.0	8.4	21	Tomato, 3-4 feet
			6.7	45	
280	5/15/44	10.2	13.2	20	Tomato, 3 feet
			9.9	49	
305	11/13/44	15.8	18.6	50	Corn, 17 days old
309	11/27/44	4.6	6.6	49	Tomato, 6-8 in. tall
311	12/ 4/44	4.0	4.2	65	Tomato, 8-10 in. tall
312	12/11/44	1.0	17.6	72	Corn, 14 days old
313	12/11/44	0.9	13.0	48	Tomato, 8-10 in. tall
317	1/ 3/45	7.8	14.2	65	Corn, 6-7 in. tall

In each experiment the same amount of plant material was used from both lots of plants and the same amount of agar was added to the extracts, so that the curvatures produced in the Avena plants are strictly comparable.

In all but one experiment given in table II, the plants in the dark have a higher hormone content than the plants in the light. Sometimes the difference is very slight, at other times very great. This is very different from the findings of AVERY, BURKHOLDER, and CREIGHTON (3), who noted that there was a depletion of the hormone in Nicotiana plants kept in the dark for some days. On the other hand, BURKHOLDER and JOHNSTON (5) found that plants removed from darkness to light lost hormone. The interpretation of all of these different results might be that hormone is destroyed by light, that in intense light more hormone is destroyed than is produced, and that in the dark the hormone is conserved. If the plant has an abundance of precursor, as has been found for tomato (6) and corn (4), there would be an increase in the hormone content in the plants in the dark (table II); if

the plant does not have an abundance of precursor, there would be a loss of hormone as the plant remained in the dark (3). Unquestionably, the hormone is indirectly dependent upon photosynthesis for its production; the longer a plant is in the dark, the less it will have, unless there is a large supply of precursor which can be transformed into the active hormone. The writer has no information on the amount of precursor in *Nicotiana*, and it can only be surmised that it is low.

#### INFLUENCE OF TEMPERATURE ON HORMONE CONTENT IN GREEN PLANTS

In making comparative studies of growth hormone, the temperature under which the plants have been growing may vary considerably if the investigation lasts for some time. The extent to which temperature influences the hormone content of the plant under study is, therefore, of considerable importance. In gaining an understanding of hormone action, temperature studies are also valuable in themselves. With these points in mind, the present study was undertaken.

As no temperature-controlled greenhouses have been available, the study was made during the winter, when the low temperature could be obtained quite readily by shutting off the radiators and opening the windows the proper amount. However, it has been impossible to keep the temperature constant; a variation of several degrees has been the rule, and on sunny days the variation was likely to be more. In most instances there has been a difference in temperature of approximately eight degrees in the two rooms; very rarely has the maximum of the lower temperature been higher than the minimum of the higher temperature during an experiment, though this has happened. The time of the year when these experiments were made is dark and cloudy in Ann Arbor, and this may have produced results different from those that would have been obtained if there had been an abundance of light.

TABLE III

GROWTH HORMONE CONTENT IN TOMATO PLANTS SUBJECTED TO DIFFERENT TEMPERATURES

EXP.	DATE	CURVATURE OF AVENA COLEOPTILE PRODUCED BY PLANTS IN			TEMPERATURE	SIZE OF PLANT
		WARM ROOM	COLD ROOM			
					°C.	
266	2/14/44	7.40	5.2 (4) *	5.4 (5)	18-21; 12-15	30-40 in.
267	3/ 6/44	5.25	3.2 (25)	.....	18-22; 12-16	Same plants as in 266
268	3/13/44	8.80	7.9 (3)	.....	18-22; 12-16	4-5 feet
269	3/20/44	7.80	9.1 (1)	.....	18-24; 12-15	20-27 in.
270	3/27/44	7.10	9.0 (1)	.....	18-24; 10-20	18-24 in.
271	4/ 3/44	6.70	5.7 (1)	10.0 (2)	19-24; 13-19	About 24 in.
274	4/24/44	5.40	6.2 (23)	.....	18-30; 15-20	Same plants as in 271

\* The number within parentheses after the curvature denotes number of days the plants were exposed to this temperature.



Tomato plants were used in all but two experiments, in which two- to three-week-old corn plants were used.

In table III are given experiments run over a period of two and a half months, using two different lots of tomato plants. In the first three experiments, older tomato plants were used, and here the plants exposed to a lower temperature had consistently less hormone than the plants kept in the warmer compartment. In the last four experiments, younger plants were used, and here almost as consistently the plants exposed to the lower temperature had a higher hormone content. In each lot of plants within an experiment the same amount of material was used, and the dilution was the same. Parts of an experiment are, therefore, comparable; but different experiments are not.

The cause of this difference is rather obscure. It does not seem possible that one group of plants, started a few weeks earlier than another, all from the same package of seeds, would behave entirely differently. Yet no external factors seem to vary enough to account for the difference in the results. It was thought that a greater amount of light in the later experiments might have increased photosynthesis or some other activity that would counterbalance effect of temperature; but a check on the weather for the weeks in question shows actually slightly less sunshine in late March and April than during the preceding weeks. One can only make the inadequate statement that the internal reactions were such that the net result was an increase in the hormone content when the plants were exposed to a lower temperature.

In order to gain more information and somewhat clarify the situation, another series of experiments was run in February of 1945. Tomato plants only slightly larger than the smaller ones in the preceding experiments and young corn plants were used. In these experiments the tomato plants were moved from a compartment of medium temperature to rooms of lower and higher temperatures to obtain, if possible, the influence of an increase as well as a decrease in temperature. The corn plants were exposed to the low temperature for varying lengths of time. Table IV gives the results. Again the lower temperature induced a decrease in hormone, and after a few days in the cold the corn plants showed no hormone, which merely means the concentration was too low to show up under the experimental conditions. Experiment 324 is very interesting as it shows an increase of hormone with an increase in temperature as well as a decrease with the lowering of the temperature. Experiments 271, 327, and 328 bring out something else; namely, that an exposure of one day to low temperature decreases the hormone, which then increases so that on the second day there may even be more than in the plants at the higher temperature. No growth studies have been made, but it is likely that at the lower temperature the hormone is used up in growth relatively more rapidly than it is formed, during the first day when growth rate may be high; later, however, the hormone formation may be somewhat higher than its use. In his extensive study of plant growth under controlled conditions WENT (18) finds "There is no correlation between day or night temperature and auxin content." His temperatures were 20° and 26.5° C.

TABLE IV  
GROWTH HORMONE CONTENT IN PLANTS SUBJECTED TO DIFFERENT TEMPERATURES

Exp.	CURVATURES OF AVENA COLEOPTILES PRODUCED BY PLANTS IN				'TEMPERATURE °C.	KIND AND SIZE OF PLANTS
	WARM ROOM		COLD ROOM			
324	16.3 (2) *	8.2 (1)	3.4 (1)	2.8 (2)	18-29; 10-18	Tomato, 23-28 in., in blossom
326	13.5	.....	7.6 (9)	.....	18-40; 10-15	Same plants as in 324
327	13.2	.....	2.9 (1)	5.0 (2)	18-40; 12-19	Corn, 19 days old
328	8.7	.....	5.2 (1)	9.5 (2)	20-26; 12-19	Same plants as in 327

\* The number within parentheses after curvature denotes number of days the plants were exposed to this temperature.

It has been the writer's experience that plants grown either in the greenhouse or outdoors during the summer have a higher hormone content than plants in the greenhouse during the winter, a difference not likely to be due to light conditions but to differences in temperature.

#### INFLUENCE OF THE PARASITE *CUSCUTA* ON HORMONE OF THE HOST PLANT

Whenever dodder penetrates the host tissue, there is a swelling as a result of increased growth activity. To see whether there was an increase in the hormone concentration coincident with this increase in growth caused by the parasite, *Cuscuta polygonorum* was allowed to attack plants. It did not grow on many of the plants supplied to it, but it produced an extensive parasitic growth on *Impatiens balsamea* and *Helianthus annuus*. These experiments were conducted in the spring of 1939, before the time the procedure of using the same quantity of plant tissue throughout an experiment had been decided upon, so that it was necessary to calculate the hormone in terms of indoleacetic acid equivalent (16); but as comparisons are made only between parts of the plant used on the same day, the error should not be serious. The material was frozen with dry ice before being ground, but the tissue was not boiled to destroy any enzymes. It has been shown by the author (7) that the amount of hormone extracted for the first 24 hours with ether from a plant finely ground is not very different from the total hormone obtained when the enzymes have been destroyed by boiling; i.e., during a period of twenty-four hours very little precursor is hydrolyzed to hormone in ether at a temperature of 15° C. Repeated extractions over several months were made, but as the first 24-hour extraction removed all the free auxin (7) from the plant material only these data will be used.

In the sunflower experiment, old and young stems infected and not infected and also the *Cuscuta* vine itself not yet in contact with the plant were used. The infected and much enlarged stem near the base of the sunflower plant contained 3.34  $\mu\text{gm. per kg.}$ , a similar uninfected portion contained 2.84  $\mu\text{gm. per kg.}$ ; the *Cuscuta* stem which had its haustoria imbedded in a younger part of the stem contained 5.24  $\mu\text{gm. per kg.}$ , while the sunflower stem to which it was attached contained only 3.96  $\mu\text{gm. per kg.}$ ; the young parasite not yet attached to the host contained 1.22  $\mu\text{gm. per kg.}$  With *Impatiens* the same relationship between plant part and auxin content held as for the sunflower, but the amount of hormone was less. It is evident that the parasitic combination increases the auxin so that the infected stem has more auxin than the uninfected; the parasite stem which has been in union with the host has the most hormone; and the young parasite not yet united with the host has the least. It is not possible to say with certainty that the parasite produces the hormone; it can certainly be stated, however, that the attack on the host plant by the parasite produces an increased amount of hormone and that continued extractions of the uninfected stem and the unattached parasite give a total precursor and hormone content of

6.05  $\mu\text{gm.}$  and 35.36  $\mu\text{gm.}$  per kg., respectively. From this one may argue that when the parasite makes organic union with the host, its precursor is hydrolyzed more actively and the hormone flows into the host to increase there the rate of growth. This line of reasoning is perhaps too far-fetched, and it might be better to say that the union of the parasite with the host sets off reactions which increase the hormone in both the host and the parasite.

THIMANN (13) considers that the nodules on leguminous roots are the result of auxin production by the *Rhizobium* spp. which infect their roots. On the other hand, other investigators (10, 11) have been led to believe that the crown gall formed by *Phytoplasma tumefaciens* is not the result of growth hormones produced by the parasite. Thus we see that there is a controversy as to the part played by growth hormones in parasitism. The present investigation definitely favors the view that when the parasite (*Cuscuta*) invades the host tissue there is an increase in hormone content which then increases the cell division and produces the typical enlargement in the host plant.

#### INFLUENCE OF CHLOROPHYLL ON AUXIN CONTENT IN CORN

When the influence of light on hormone was investigated, corn was germinated in the greenhouse and in the darkroom connected to the greenhouse.

TABLE V

AUXIN CONTENT OF GREEN AND ETIOLATED CORN SEEDLINGS

EXP.	DATE	CURVATURE IN AVENA COLEOPTILES PRODUCED BY PLANTS	
		GREEN	ETIOLATED
297	8/ 7/44	7.9	16.4
301	8/14/44	5.1	14.7
303	8/29/44	0.0	4.3

The plants grown in the darkroom were, of course, etiolated; because it was realized that they had not only been subjected to the lack of light but were also without chlorophyll, these plants were not used in the study of the effect of light. They were, however, compared with those grown in the light, and it was found that the etiolated plants contained much more hormone than the green plants (table V).

Because of the two-factor variable it was not possible to explain the large hormone content in these chlorophyll-less plants, but some earlier experiments with albino corn were recalled. In these experiments (exps. 255, 262, table VI) the albino plants also had a much higher hormone content than the green plants. Further experiments with other segregations gave similar results.

How chlorophyll functions to decrease the hormone has not been investigated. Not enough albino seeds have been available to grow plants in the dark and in light. The most obvious explanation is that chlorophyll acts as a photosensitizer, and the exposure to light thus destroys some of the hor-

TABLE VI

AUXIN CONTENT IN GREEN AND ALBINO CORN SEEDLINGS

EXP.	DATE	CURVATURES IN AVENA COLEOPTILES PRODUCED BY PLANTS		REMARKS
		GREEN	ALBINO	
255	11/24/43	3.3	12.2	Mains' corn
262	1/12/44	9.1	18.7	Mains' W18 and W18 (?)
304	11/ 6/44	0.0	5.9	Emerson's 43-85-39 (yellow)
306	11/15/44	0.3 +	10.5	Emerson's 43-85-15 (white)
310	11/27/44	3.7	15.6	Mains' corn

more produced by the plant. The greater hormone content in the etiolated plants may be partly responsible for the greater growth of plants in the dark; however, the albino plants grown in the light are usually slower in developing than the green plants from the same culture.

#### COMPARISON OF THE AMOUNT OF HORMONE IN DWARF AND TALL CORN PLANTS

In 1935, and later in 1938, VAN OVERBEEK (14, 15) considered that dwarf corn was dwarf because the plants produced less and destroyed more hormone than did the tall plants. During the past year the writer has had available dwarf and tall corn seedlings produced from the same ear. Plants about three weeks old from a number of different segregations have been used.

The writer would hesitate to say that the dwarf seedlings have more hormone than the tall seedlings; he would, however, be justified in saying (from these three experiments, which represent plants from at least eight different crosses) that they have at least as much hormone as the tall plants. In another paper in preparation, the matter of inhibitors will be discussed. It may be that the relation between dwarfness and low hormone content found by VAN OVERBEEK was just a coincidence and not cause and effect.

#### INFLUENCE OF AGE OF AN ORGAN UPON ITS HORMONE CONTENT

In several experiments in which tomato plants 6 to 8 feet tall were used, leaves from different regions were compared as to the amount of hormone

TABLE VII

HORMONE IN DWARF AND TALL CORN PLANTS OBTAINED FROM THE SAME PARENTS

EXP.	DATE	CURVATURE IN AVENA COLEOPTILES PRODUCED BY PLANTS	
		TALL	DWARF
311	11/29/44	6.6	10.6
318	1/ 8/45	9.0	6.3
325	1/31/45	1.2	5.7

they contained. Young leaves near the tip of the plant were always the richest in hormone, and the lowest leaves were the poorest even when they still appeared perfectly healthy. This finding led to a study of the influence of the age of a plant and the position of the growing region on the auxin content of a particular plant. Tips of the main stem with a few young leaves were taken from plants only 12 inches tall and from plants 40 inches tall (a difference in height which means about 2 to 3 months difference in the age of the plant). From the group of the older plants the tips of branches also were collected and examined separately. Experiment 155 will suffice to illustrate the results. The concentration of hormone in the tips of the 12-inch plants was 6.5  $\mu\text{gm. per kg.}$ ; in the apical tips of the 40-inch plants it was 3.8  $\mu\text{gm. per kg.}$ ; and in the tips of the lateral branches of the same plants it was 3.5  $\mu\text{gm. per kg.}$  Thus it is shown that younger plants have more hormone in the actively growing region than do older plants, and that all growing regions on the same plant have the same amount of hormone. The latter point should be investigated more fully.

#### DISTRIBUTION OF GROWTH HORMONE IN THE FLOWER CLUSTER OF THE TOMATO

In parthenocarpic work with chemicals it has always been least difficult to induce fruiting in the first blossom of a cluster of the tomato. The ques-

TABLE VIII

HORMONE CONTENT OF THE OVARIES FROM FLOWER BUDS SITUATED IN DIFFERENT POSITIONS IN A FLOWER CLUSTER. THE HORMONE CONCENTRATION IS GIVEN IN INDOLEACETIC ACID EQUIVALENTS OF MICROGRAMS PER KILOGRAM

EXP.	DATE	LOCATION OF BUD IN CLUSTER		
		1	2	3, 4
124	7/19/41	50.2	18.8	11.7
177	6/22/42	12.0	7.5	.....
180	6/29/42	27.7	11.6	.....

tion as to the relative amount of hormone in the different buds has been raised many times. Not only does the first bud set most readily, but it invariably produces the largest fruit. GUSTAFSON and HOUGHTALING (9) have shown that there is a relation between the size of the ovary in the flower and the size of the fruit set, and that the ovary in the first flower in a cluster is the largest. Is food, as these investigators thought, the only factor in the production of a large ovary in the first flower, or is there also a hormonal difference?

Flower buds one and two were always obtained from the same cluster and buds three and four, when used, came from an older cluster; i.e., one in which buds one and two had already opened. The data show that there is a difference in hormone content of the ovaries from flower buds located in different positions in a cluster. The first one formed has the highest concen-



tration and the second the next. The readiness with which the first flowers in a cluster set fruit may be connected with this high hormone content although further development does also depend on such nutritional factors as food and water.

### Discussion

In this series of experiments, growth hormone concentration tends to correlate with rate of growth. That is, those conditions like rich soil, high temperature, and moderate intensity of light, which we know favor vegetative growth, are also favorable to the production of growth hormone. Even intumescences produced by parasites like *Cuscuta* can be considered as a product of the high growth hormone concentration obtained in the host as a result of the parasitic attack.

Like the external factors, internal factors also have an influence on hormone production. In previous experiments (8) the author has related fruitfulness in Montmorency cherry to the amount of hormone present in the flower buds; in the present investigation this idea is further strengthened by finding that those buds in the inflorescence of the tomato which most readily produce fruits also have a high hormone content. This statement does not minimize the importance of food, water, enzymes, or accessory growth factors; it merely emphasizes the importance of the hormone.

Differences in chromosome number have previously (8a) been shown to be associated with hormone production; in this series of experiments it is shown that chromosomal differences may also influence hormone content of a plant. Corn plants lacking the ability to produce chlorophyll produce, or at least contain, more growth hormone than those plants that produce chlorophyll. It remains to be determined whether or not light is a factor here. Perhaps chlorophyll is an agent through which light destroys some of the hormone produced by the green plant. The experiments with the etiolated corn plants seem to warrant such an assumption, but only by growing albino and chlorophyll-producing plants in the dark can this be determined. Not enough seeds have been available to do this at the present time. Whatever the mechanism, the fact remains, that corn plants unable to produce chlorophyll contain more hormone than plants that do produce chlorophyll.

It is a well-known fact that as a plant grows older its physiological activities change and that young shoots of the same species, but from individuals of different age, may respire or photosynthesize at different rates and that they are even able to strike roots at different rates. The same is seen to be true for growth hormones; shoot tips from young plants contain more hormone than those from older plants. It seems to the writer that whatever else this series of experiments may have done it ties hormones more closely to physiological phenomena and makes them more definitely members of the family.

### Summary

It has been found that poor mineral nutritive conditions, low tempera-

ture, and high light intensities lower the growth hormone content in plants investigated (tomatoes and corn).

Stems of *Helianthus annuus* and *Impatiens balsamea* parasitized by *Cuscuta polygonorum* have a higher growth hormone content than stems not so parasitized.

Corn seedlings devoid of chlorophyll, either because they have been grown in the dark or because of their genetic constitution, have more growth hormone than do similar plants with chlorophyll.

As a part of a plant, or the plant as a whole, ages its growth hormone content becomes less.

Flower buds formed first in an inflorescence of the tomato have more hormone and are more likely to set fruits than those formed later.

The dodder seeds were obtained from Dr. H. L. Dean at the State University of Iowa, Iowa City, Iowa.

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# THE LACK OF SCION EFFECT ON ROOT QUALITY OF *DERRIS ELLIPTICA*<sup>1</sup>

MERRIAM A. JONES AND WILLIAM C. COOPER

## Introduction

In rotenone-bearing crops, such as *Derris elliptica* (Wall.)<sup>2</sup> Benth., in which the root is valued for insecticidal purposes, the yield of root as well as the rotenone content is important. Obviously yield could not be sacrificed to obtain high rotenone percentage, nor could high yield compensate for low rotenone content. A maximum yield of root of uniformly high rotenone content may be considered as a goal.

Of the several types of *D. elliptica* available at this station, one, the St. Croix variety, is characterized by a high yield of root markedly low in rotenone. Another, Changi No. 3, is characterized by a lower yield of root which is relatively high in rotenone. A third variety, the Sarawak Creeping, occupies an intermediate position with regard to rotenone content.

The extent to which grafting may modify the yield and rotenone content in Derris roots has never been investigated. The possibility of increasing the rotenone content of the more vigorous St. Croix roots by grafting on scions of the Changi No. 3 seemed promising. Likewise, St. Croix tops on Changi No. 3 roots might increase the yield of roots of the latter. An experiment testing these premises is here reported, the results of which, although negative with regard to the anticipated scion effect, are of interest.

A hypothesis under test in this experiment concerned the possibility of the leafy part of the plant supplying some factor, perhaps an intermediate compound, similar to but simpler than rotenone, which was necessary for the completion of the synthesis of rotenone in the roots. This was suggested by the fact that compounds of the rotenone group are chemically related to compounds of the flavone group, of which at least two occur in the leaves of derris.<sup>2</sup> Rotenone itself (I) can be considered as an isoflavanone nucleus (II) in which a methoxyl group on 6' is condensed with a hydroxyl group on 2 to result in a bridge. On 7 and 8 there is a pentose residue and on 3' and 4' methoxyl groups.

That compounds related to rotenone exist in the root in glycosidic combination (probably acylated) is indicated by the fact that derris resin must be hydrolyzed by mild alkali treatment before crystalline deguelin can be isolated.

## Methods

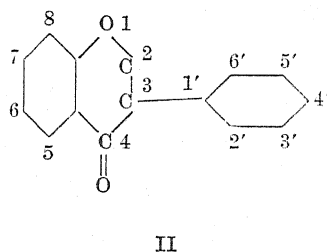
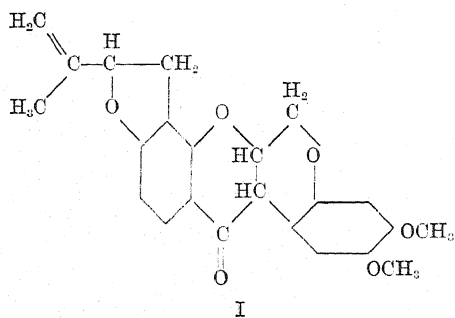
Reciprocal grafts of high-rotenone Changi No. 3 and low-rotenone St. Croix were made by whip grafting leafless stem cuttings of the 2 varieties.

<sup>1</sup> This work was done at the Federal Experiment Station, Office of Experiment Stations, Agricultural Research Administration, U. S. Department of Agriculture, Mayaguez, Puerto Rico, and was a cooperative project between this Station and the Office of Foreign Agricultural Relations, U. S. Department of Agriculture.

<sup>2</sup> Jones, M. A. Unpublished data.

Control grafts consisting of each type grafted on itself were made at the same time. Ungrafted control cuttings of each variety were also included in the experiment making a total of 6 treatments each consisting of 10 grafts. The cutting material used was taken from leafy vines with mature brownish bark. Since all material of each type was taken from an individual plant, each resulting group of plants was a single clone.

The grafts were prepared in the laboratory and tied with string, no wax or bandage being used. Cuttings were placed in a sash-covered sand bed in the greenhouse at high humidity for 6 weeks; during this time the unions healed and the stocks rooted. They were then transplanted to nursery beds in the field, each row constituting one treatment. During the time the plants were growing in the field as well as in the propagating frame, the spacing was 2 inches between plants in rows 4 inches apart. The unions were always kept clear of the soil in order to avoid separate rooting of the scion.



Eleven months after grafting, the tops were removed and the roots of each plant excavated as completely as possible. The roots were then divided into two size groups, those smaller than 5 mm. diameter and those 5 mm. and over.

To obtain a measure of quality of the separate small samples, the dry root was weighed and ground through the 20- and 60-mesh sieves of a micro Wiley mill, and colorimetric determinations were made for rotenone plus rotenoids in each sample (1), after which suitable composites were made for an additional colorimetric test and rotenone analyses by the A.O.A.C. method (2).

A similar experiment was performed simultaneously in which grafts were made between Sarawak Creeping and St. Croix.

### Results

The roots of the St. Croix stocks tended to be of large diameter, some over 10 mm., with few fine roots. The root system of the Sarawak Creeping variety stock tended to be larger than that of Changi No. 3 and considerably more branched than that of the St. Croix. At the time of harvest it was noted that the bark of some roots of the Changi variety contained a reddish pigment whether the scion was Changi No. 3 or St. Croix.

Chemical analyses of the roots, summarized in table I with the survival and yield data, show no effect of scion on yield of root or on rotenone content.

TABLE I  
EFFECT OF GRAFTING VARIETIES OF *Derris elliptica* ON THE YIELD AND QUALITY OF THE ROOT

GRAFTS	PLANTS SURVIVING	YIELD, DRY ROOT PER PLANT		COLORIMETRIC, ROTENONE PLUS ROTENONDS				ROTENONE*	RATIO ROTENONE TO ROTENONE PLUS ROTENONDS
		RANGE	AVERAGE	CONTENT		PER PLANT			
				RANGE	WEIGHTED AVERAGE	RANGE	WEIGHTED AVERAGE		
	no.	gm.	gm.	%	%	gm.	gm.	%	
<i>scion/stock</i>									
Changi No. 3 ungrafted .....	10	0.2 - 6.95	3.05	8.9-12.6	11.0	0.03-0.87	0.33	5.7 and 6.9	0.52
Changi No. 3/Changi No. 3 .....	5	0.8 - 11.15	4.47	3.2- 9.9	8.5	0.03-1.10	0.38	4.5	0.55
St. Croix/Changi No. 3 .....	10	0.5 - 8.4	3.30	7.3-11.6	9.4	0.05-0.89	0.31	5.2 and 5.9	0.56
St. Croix ungrafted .....	10	1.85-26.45	8.82	1.2- 5.9	2.9	0.05-0.54	0.26	0.9 and 1.4	0.45
St. Croix/St. Croix .....	8	1.4 - 15.5	5.13	2.4- 4.2	2.9	0.05-0.38	0.15	1.1	0.34
Changi No. 3/St. Croix .....	9	0.45- 8.85	3.37	2.4- 4.6	3.5	0.02-0.27	0.12	1.1 and 1.5	0.35
Sarawak Creeping/St. Croix† .....	3	3.65-11.8	6.48	1.1- 4.1	2.1	0.13-0.15	0.14	1.0	0.45
St. Croix/Sarawak Creeping† .....	3	3.0 - 13.25	7.87	4.0- 4.9	4.3	0.12-0.53	0.33	2.7	0.57

\* Two figures are reported for those treatments of which there was sufficient material to prepare two samples for rotenone analysis.

† The controls of these two treatments did not survive.



Another striking feature in the data was the wide variation among plants of the same clone, especially with regard to quality as measured by the colorimetric test or rotenone content. No correlation could be found between quality and yield in any of the treatments.

The ratio of rotenone to rotenone plus rotenoids in the St. Croix root material was low, about 0.35 to 0.45; that of the higher quality Changi No. 3 and Sarawak Creeping had a ratio of 0.50 to 0.55.

### Discussion

It was not considered necessary to transplant the experimental material to the field to await maximum root and top development because the effect of scion on stock is a definite one; that is, either there is an effect or there is none. Any increase or decrease of rotenone in the stock caused by the grafted scion would be apparent in the young as well as in the more developed plant. In this experiment rotenone was found in comparatively large amounts in roots of Changi No. 3 and in much smaller concentrations in roots of the St. Croix regardless of tops used. Therefore, although growth took place in these plants and the scion obviously furnished food to the stock, no effect on rotenone content could be demonstrated. The grafted and ungrafted plants contained rotenone in concentrations characteristic of the stock. Thus it appears that the leafy top of the plant probably does not control production of rotenone by furnishing a hypothetical precursor which would be transported to the roots and deposited there as rotenone. The synthesis of rotenone would therefore appear to be confined principally to the root system. This phenomenon resembles that found in reciprocal grafts among tomato, *Datura*, and tobacco (3) in which it was found that the root was the center of synthesis of some plant poisons.

### Summary

Grafting between high- and low-rotenone varieties of *Derris elliptica* (Wall.) Benth. showed that there was no effect of top on root as far as yield of root and percentage of rotenone were concerned. This indicates that the root system is not dependent on the top for a unique precursor of rotenone.

The authors are indebted to Mr. CALEB PAGAN, employed by the War Emergency Program of the Government of Puerto Rico, for his aid in part of the chemical analyses.

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## EFFECT OF AUXINS ON THE ACTION OF DIASTASE *IN VITRO*

H. C. EYSTER

(WITH TWO FIGURES)

It has been shown by EYSTER (2) that indole-3-propionic acid both retards the action of isolated diastase on soluble starch and accelerates the action of diastase associated with charcoal by releasing the adsorbed diastase from the charcoal. In this paper the actions of indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid are compared both in their effects on the action of isolated diastase and in their effects on the action of diastase adsorbed upon activated charcoal.

### Methods and materials

The technique used here is the same as that described by EYSTER (2), wherein the enzyme, diastase of malt Merck (medicinal, U.S.P. IX), was adsorbed upon particles of activated charcoal. The effectiveness of diastase as a hydrolytic agent for soluble starch was measured by determining the time required to digest a given amount of soluble starch past the last iodine-staining stage.

Norit A was the type of activated charcoal used. It was obtained by the University of South Dakota Chemistry Department from the Pfanstiehl Chemical Company in Waukegan, Illinois. Norit by definition (3) is a purified charcoal made from birchwood and is used to decolorize and deodorize syrups, oils, or pharmaceutical products. Inasmuch as the Norit A showed variations in adsorptive capacity with changes in the moisture content of the atmosphere, it was dehydrated by placing it in an oven at 107° C. for 24 hours and then stored in a desiccator with dry  $\text{CaCl}_2$ . Since there was the possibility that the adsorptive capacity of the charcoal may be affected by  $\text{CO}_2$  content of the atmosphere, KOH was also placed in the desiccator. The temperature of the solutions was always kept close to 25° C. by the use of a water bath. All of the special chemical compounds used in this research were purchased from Eastman Kodak Co., Rochester, N. Y.

### Results

To test the effect of the auxins on the action of isolated diastase 25 and 50 p.p.m. of each auxin were used in separate mixtures. The control mixture contained 50 ml. of 1 per cent. soluble starch, 49 ml. of distilled water, and 1 ml. of 1 per cent. diastase of malt. The mixtures having 25 p.p.m. auxin were made up of 50 ml. of 1 per cent. soluble starch, 5 ml. of a solution containing 500 p.p.m. auxin, 44 ml. of distilled water, and 1 ml. of 1 per cent. diastase of malt. The mixtures having 50 p.p.m. auxin were prepared by taking 50 ml. of 1 per cent. soluble starch, 10 ml. of a solution containing 500 p.p.m. auxin, 39 ml. of distilled water, and 1 ml. of 1 per cent. diastase of malt.

The time required for the digestion of the soluble starch past the last iodine-staining stage, and the pH of each mixture was determined. The rate of digestion was determined by the simple  $I_2KI$  test, using a dilute solution. A glass electrode pH meter was used to determine the pH of each mixture.

The results are recorded in table I giving the effect of the respective auxins on enzyme action proper. Indole-3-propionic acid has the least retarding effect on the action of isolated diastase. It is followed in the order of effectiveness by indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid. Alpha-naphthalene acetic acid is the most active of the four auxins used. If the control is arbitrarily assigned the value of 100 for its digestive rate, the digestive rates of the mixtures with 25 p.p.m.

TABLE I

EFFECT OF FOUR DIFFERENT AUXINS ON THE ACTION OF ISOLATED DIASTASE; I.E., DIASTASE NOT ASSOCIATED WITH ACTIVATED CHARCOAL

MIXTURES	TIME REQUIRED FOR DIGESTION PAST LAST IODINE STAINING STAGE	PERCENTAGE OF CONTROL*	pH
	<i>min.</i>	<i>%</i>	
Control .....	125	100	5.9
Indole-3-propionic acid, 25 p.p.m. ....	132	95	5.5
Indole-3-butyric acid, 25 p.p.m. ....	150	83	5.2
Indole-3-acetic acid, 25 p.p.m. ....	165	76	5.3
Alpha-naphthalene acetic acid, 25 p.p.m. ....	175	71	4.7
Indole-3-propionic acid, 50 p.p.m. ....	145	89	5.0
Indole-3-butyric acid, 50 p.p.m. ....	160	78	4.9
Indole-3-acetic acid, 50 p.p.m. ....	170	74	4.7
Alpha-naphthalene acetic acid, 50 p.p.m. ....	185	68	4.5

\* Time for control divided by time for experimental test, expressed in percentage.

of indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid are calculated to be 95, 83, 76, and 71, respectively. The effects of 50 p.p.m. of each of the auxins are greater.

If pH and the rapidity of digestion are plotted as in figure 1, it can be seen that there is a direct relationship. The sole cause of the difference in the effect of the four auxins on isolated diastase seems to result from differences in their hydrogen ion concentration. Corroborating evidence was obtained by using very dilute solutions of acetic acid to produce a series of pH levels and then to test the rapidity of diastase action at those pH levels. The lower the pH, the slower was the diastatic activity on soluble starch. Buffer solutions were not used to stabilize pH because buffer salts are not neutral in their effects on enzyme action.

To determine the effects of auxins on the action of diastase adsorbed upon activated charcoal, the control mixture contained 50 ml. of 1 per cent. soluble starch, 45 ml. of distilled water, 5 ml. of 1 per cent. diastase, and 1 gm. of Norit A; mixtures with 25 p.p.m. auxin had 50 ml. of 1 per cent. soluble

starch, 5 ml. of a solution containing 500 p.p.m. auxin, 40 ml. of distilled water, 5 ml. of 1 per cent. diastase, and 1 gm. of Norit A; and mixtures with 50 p.p.m. auxin had 50 ml. of 1 per cent. soluble starch, 10 ml. of a solution containing 500 p.p.m. auxin, 35 ml. of distilled water, 5 ml. of 1 per cent. diastase, and 1 gm. of Norit A. In preparing the mixtures, all of the ingredients except diastase and Norit were mixed; the diastase was then added. The mixtures were shaken a bit and the Norit added quickly with enough shaking to thoroughly mix it with the other constituents. Every one-half

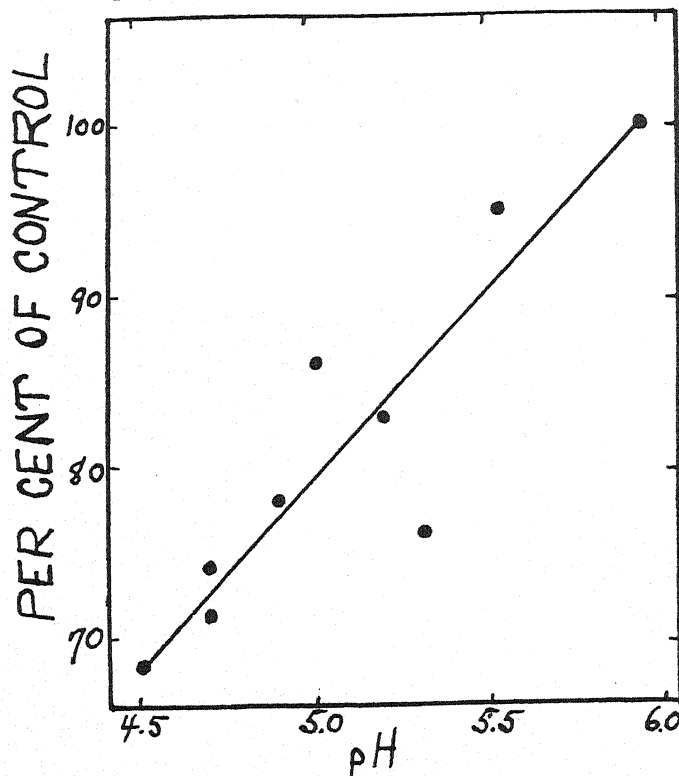


FIG. 1. Interrelation of pH and rate of digestion by diastase. In the range between pH 4.5 and 6.0, as the pH increases the rate of digestion increases by a seemingly constant acceleration.

hour the solutions were shaken, and 2 ml. of each mixture were filtered through two thicknesses of fine textured filter paper and tested with one to several drops of a dilute solution of  $I_2KI$ . One drop was used during the initial stages of the experiment, and two to five drops were used later on. The data are presented graphically in figure 2. The results indicate the same order of effectiveness as was obtained in the previous experiment. Indole-3-propionic acid is the least active and alpha-naphthalene acetic acid is the most active. The periodic tests every 30 minutes indicated that, at the start, the least active auxin appeared the most active, and the most active auxin appeared the least active. At the start, it appears that most of the

diastase is adsorbed and thereby inactivated. The small portion which is still free to act would then be impeded least by the least active auxin. Digestion in the control is slowest and possibly is impeded because the diastase is more thoroughly adsorbed there than in mixtures containing auxins. Auxins release the diastase from the activated charcoal. The interaction of auxins and diastase adsorbed on charcoal apparently involves two phases, accounting for a change in the rate of digestion. At first the auxin interaction is dominated in a phase characterized by the retardation of enzyme action proper of the unbound diastase. Later the auxin interaction is dominated in a phase characterized by the release of bound diastase and a consequent acceleration in diastase activity. The slowness of the mixture with indole-3-propionic acid, at the end, is probably due to its extreme ineffectiveness in releasing diastase from the activated charcoal and its simultaneous retardation of enzyme action proper. It appears that the release of diastase by auxins consists of a displacement of auxins on the charcoal surface by hydrogen ions. The least active auxins appear to displace very little diastase leaving an abundance of free auxin to retard the small amount of free enzyme.

The tests with iodine, to show the relative amounts of soluble starch still undigested, were very consistent, clear-cut and definite. While the total time required for digestion, past the last iodine-staining stage in the four auxin and one control mixtures, may not be significant, there was a consistent and orderly trend in the course of digestion in each solution.

The pH of all of these charcoal mixtures regardless of the auxin present is the same—pH 8.2. Control solutions without any auxin also have the same pH. It is the belief of the author that hydrogen ions here too play a dominant influence in the release of auxins from the surface of charcoal particles and that there are pH contours closely surrounding the charcoal particles. While the pH of the free fluid between the charcoal particles is 8.2, the adsorbed layers on the surface may have lower and lower pH's with the lowest pH on the immediate surface. The pH on the immediate surface is expected to vary with the auxin used, and this, if true, would explain the variation in the release of auxin by displacement by various auxins and by various concentrations of the same auxin. There is evidence that ions other than hydrogen influence adsorption of charcoal and enzymes (1) and so, with the great diversity of numerous growth substances, the interaction of growth substances and enzymes need not in all cases be explained on the basis of hydrogen ion concentration effects.

The rate at which different auxins release diastase from activated charcoal was determined with another experiment. One gm. of Norit A was mixed with 35 ml. of distilled water and 5 ml. of 1 per cent. diastase and  $2\frac{1}{2}$  hours allowed for fairly complete adsorption of the diastase. Immediately at the end of  $2\frac{1}{2}$  hours, 50 ml. of 1 per cent. soluble starch, 5 ml. of a solution containing 500 p.p.m. auxin and 5 ml. of distilled water were added. This gave the final mixtures auxin concentrations of 25 p.p.m. To other mix-



tures, 50 ml. of 1 per cent. soluble starch and 10 ml. of a solution containing 500 p.p.m. auxin were added to produce mixtures with concentration of 50 p.p.m. Digestive tests with  $I_2KI$  were made on these solutions at the end of 24, 31, 44, 48, and 50 hours. The ones with 25 p.p.m. indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid are of special interest. Twenty-four-, 31-, 44-, and 48-hour tests with 25 p.p.m. and 50-hour tests with 50 p.p.m. gave significant results which conform with the results of the preceding experiment.

In the 24-hour test using 25 p.p.m. auxin, the order from greatest amount of digestion to the least was (1) indole-3-propionic acid, (2) indole-3-butyric acid, (3) indole-3-acetic acid, (4) alpha-naphthalene acetic acid, and (5) control. Seven hours later (31-hour test) the order was (1) indole-3-acetic acid, (2) indole-3-propionic acid, (3) indole-3-butyric acid, (4) alpha-naphthalene acetic acid, and (5) control. The 44-hour test had (1) indole-3-acetic acid, (2) alpha-naphthalene acetic acid, (3) indole-3-propionic acid, (4) indole-3-butyric acid, and (5) control; while the 48-hour test had (1) alpha-naphthalene acetic acid, (2) indole-3-acetic acid, (3) indole-3-propionic acid, (4) indole-3-butyric acid, and (5) control. In the 50-hour test using 50 p.p.m. auxin, the order from the greatest amount of digestion to the least was (1) alpha-naphthalene acetic acid, (2) indole-3-acetic acid, (3) indole-3-butyric acid, (4) indole-3-propionic acid, and (5) control.

### Discussion

There is much diversity of results in the literature on the comparative effectiveness of auxins on plant growth. This can possibly be explained on the basis of variations of concentrations used, the time factor, and the influence of light in some cases. The more active auxins, *in vitro*, appear to be less effective in greater concentrations whenever the time is comparatively brief. This is clearly indicated in figure 2. The comparative digestive stages in the auxin-diatase-charcoal soluble starch mixtures vary continually, with the less active auxins more rapid at first followed and surpassed by the more active auxins. While there is considerable diversity, the literature contains records in the majority of which the order of comparative effectiveness is the same as that demonstrated in this paper by the charcoal technique. MARMER (6) found that the concentrations causing 50 per cent. reduction in growth of primary root of Marquis wheat seedlings at pH 4.6 were: indole-3-acetic acid, 0.012 mg. per liter; indole-3-butyric acid, 0.055 mg. per liter; and indole-3-propionic acid, 0.250 mg. per liter. The exact position of alpha-naphthalene acetic acid in the series is indicated by MACHT and GRUMBEIN (5). They found that the growth of roots of *Lupinus albus* seedlings was definitely retarded by long exposures to alpha-naphthalene acetic acid, indole-3-acetic acid, and indole-3-butyric acid. The former was the most effective and the latter least effective of the three auxins used. ZIMMERMAN'S (7) data indicate the same order of effectiveness. For total root lengths of *Elodea* at 16 days using

a concentration of 1 p.p.m., KING (4) found that the order of decreasing magnitude of response was indole-propionic acid, indole-butyric acid, indole-acetic acid, 1-tryptophane, phenyl-acetic acid, control, vitamin B<sub>1</sub>, and naphthalene acetic acid.

From the *in vitro* experiments described in this paper, it might be concluded hypothetically that there are two phases in the interaction of auxins on charcoal-adsorbed diastase: phase I, release of enzyme in whole or in part from the charcoal; and phase II, effect on enzyme action proper whereby reactants are adsorbed upon the surface of the enzyme and are made to combine to form end products, apparently because the reactant molecules are subjected to gigantic adsorptive pressures as well as by being

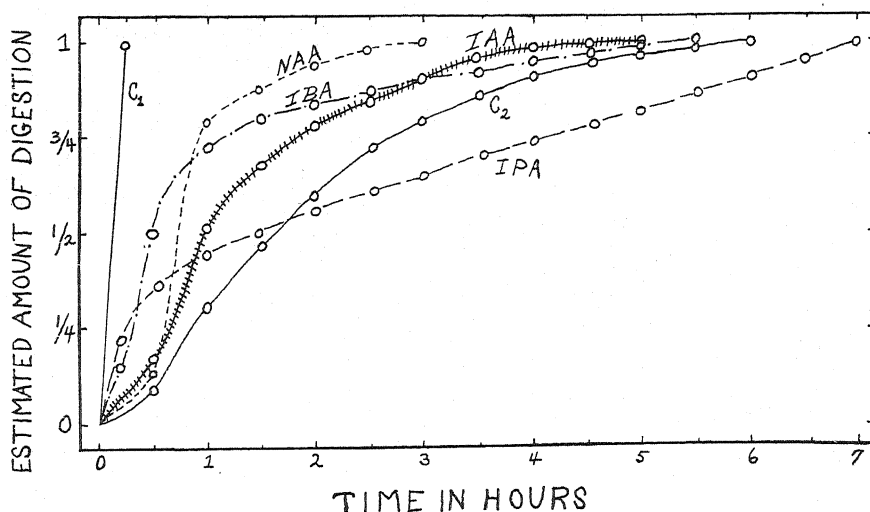


Fig. 2. Effect of auxins on the action of diastase adsorbed upon activated charcoal. The digestion was followed colorimetrically up to and including the last iodine-staining stage at half-hour intervals. C<sub>1</sub>, control in the absence of activated charcoal; C<sub>2</sub>, control in the presence of activated charcoal; NAA, with alpha-naphthalene acetic acid; IBA, with indole-3-butyric acid; IAA, with indole-3-acetic acid; and IPA, with indole-3-propionic acid.

brought into closer proximity. If the influence of an auxin or factor dominates in phase I, then the action of the enzyme is accelerated because more unbound enzyme is made available, and the factor can logically be called a "growth-promoting factor" or "growth substance." If the influence of the same auxin or factor dominates in phase II, then the action of the enzyme is decelerated, and the factor can be called a "growth-inhibiting factor" or "growth inhibitor." In each phase, adsorption is an important process involved.

The interaction of auxins and diastase on charcoal surfaces may well be the way in which auxins and enzymes interact on the surfaces of cellular colloids.

## Summary

The interaction of auxins and diastase of malt was investigated *in vitro*, both in isolated and activated charcoal systems. Indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid were used in concentrations of 25 and 50 p.p.m. at 25° C. In the activated charcoal systems 1 ml. of 1 per cent. diastase was adsorbed upon 1 gram of Norit A. The influence of the auxins on diastase, in isolated systems, was measured by determining the time required to digest a given amount of soluble starch past the last iodine-staining stage. The influence of auxins on diastase in activated charcoal systems was measured by regular half-hour comparative color tests with I<sub>2</sub>KI. Auxins retarded the action of isolated diastase and accelerated the action of diastase adsorbed on charcoal in the same order. The order of effectiveness from least active to most active was: indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid. The effectiveness of the auxins was correlated with pH.

It might be concluded hypothetically that there are two phases in the interaction of auxins on charcoal-adsorbed diastase: phase I, release of enzyme in whole or in part from the charcoal; and phase II, effect on enzyme action proper. If the influence of an auxin or factor dominates in phase I, then there is increased enzyme action, and the factor can logically be called "growth-promoting." If the influence of the same auxin or factor dominates in phase II, then there is a decrease in enzyme action, and the factor can just as logically be called "growth-inhibiting." The interaction of auxins and diastase on charcoal surfaces may well be the way in which auxins and enzymes interact on the surfaces of cellular colloids.

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# EFFECTS OF IRON ON CERTAIN NITROGENOUS FRACTIONS OF *ANANAS COMOSUS* (L.) MERR.<sup>1</sup>

C. P. SIDERIS AND H. Y. YOUNG

(WITH SIX FIGURES)

## Introduction

The effects of ammonium- vs. nitrate-nitrogen in solution cultures on the nitrogenous fractions in the tissues of *Ananas comosus* (L.) Merr. were reported in previous publications (31, 32). Data on the effects of plus- or minus-iron cultures supplied with nitrate- or ammonium-nitrogen on the growth and the tissue content of various ash constituents, chlorophyll, acidity, ascorbic acid, and carbohydrates of *A. comosus* were also reported in two former papers (29, 33).

This paper is concerned with the effects of plus- vs. minus-iron cultures in association with those of ammonium- or nitrate-nitrogen on the nitrogenous fractions of the tissues of *A. comosus*.

## Methods

Methods employed for growing the experimental plants were described in the first paper of this series (33) while the procedure for sectioning and preparing them for chemical analysis was reported in earlier publications (30, 31). In brief, the plants were grown for 12 months in 4-gallon porcelain containers, then harvested and sectioned for analysis. The nutrient solutions were aerated constantly and changed regularly at 2-week intervals.

The nitrogenous fractions studied were: total nitrogen, sap-soluble inorganic-N, nitrate-N, ammonium-N, sap-soluble organic-N, amide-N, alpha-amino-N, mono-amino-N, basic-N, peptide-N, proteose-peptone-N, and protein-N.

Nitrate nitrogen was determined by the phenoldisulfonic acid method. Total nitrogen, including nitrates, was determined according to the methods of PUCHER *et al.* (26) and VICKERY (35).

The procedure for the separation of the different fractions was as follows: The pulp from 50 grams of fresh plant tissues, thoroughly ground in a mortar with quartz sand, was placed in a 100-ml. flask to which was added 1 ml. of glacial acetic acid and water to the mark; the mixture was heated to boiling and then centrifuged or filtered. The centrifugate or filtrate was removed and the residue washed twice with 25 ml. of hot 1 per cent. acetic acid. The centrifugate from both washings was combined; the residue was employed for the determination of insoluble or protein nitrogen. Total nitrogen in the centrifugate after subtraction of nitrate and proteose-peptone nitrogen was classified as soluble organic nitrogen. A 10-ml. aliquot of the

<sup>1</sup> Published with the approval of the Acting Director as Technical Paper no. 155 of the Pineapple Research Institute, University of Hawaii.

centrifugate was employed for the determination of alpha amino nitrogen by the Van Slyke method after subtracting ammonium nitrogen (values ranging from 0.002 to 0.020 milligrams per gram of fresh tissue) which was determined by the method of PUCHER, VICKERY, and LEAVENWORTH (27).

The centrifugate was treated with 10 ml. of lead acetate (10 gm. of lead acetate in 90 ml. of 1.5 per cent. acetic acid) and allowed to stand overnight. The precipitate was separated from the supernatant liquid by centrifugation and washed with 20 ml. of 80 per cent. alcohol. It was analyzed for nitrogen by the Kjeldahl process, using selenium oxychloride as catalyst. This fraction was designated as the "soluble protein" fraction and was probably identical with the so-called "proteose-peptone" fraction, as evidenced by its precipitation with lead acetate and tannic acid (23).

The filtrate or centrifugate from the above operation, containing also the alcoholic washings, was treated with 10 ml. of mercuric acetate (10 gm. of mercuric acetate in 100 ml. of a 2.0 per cent. acetic acid) without removal of the excess lead of the former treatment. The removal of such lead in the form of lead sulfide or lead sulfate, either with hydrogen sulfide gas or with sulfuric acid and precipitation of excess sulfuric acid with  $\text{Ba}(\text{OH})_2$ , was omitted because it removed some nitrogen by adsorption. The mixture was allowed to stand overnight, and the precipitate was removed either by centrifugation or filtration and then washed with 20 ml. of 80 per cent. alcohol. The precipitate was analyzed for nitrogen by the Kjeldahl process as mentioned above. This fraction of nitrogen was classified as peptide (17).

The centrifugate or filtrate from the above operation was treated with 3 ml. of concentrated  $\text{H}_2\text{SO}_4$ , and the precipitate, consisting mostly of  $\text{PbSO}_4$ , was removed by centrifugation and subsequently washed twice with  $\text{H}_2\text{O}$ . The precipitate was discarded, and the centrifugate was made to 200 ml., treated with 4 ml. of concentrated  $\text{H}_2\text{SO}_4$ , heated over a boiling water bath for 3 hours, then made alkaline for the distillation of ammonia under the conditions recommended by PUCHER, VICKERY, and LEAVENWORTH (27) for amide nitrogen.

After the removal of amide nitrogen the mixture was acidified with  $\text{H}_2\text{SO}_4$  and treated with phosphotungstic acid for the separation of mono-amino-nitrogen from basic-nitrogen as described previously (30).

No fractionation of protein nitrogen was attempted because somewhat comparable data had already been obtained in a former study (31).

The statistical significance of the difference of the items between the F and O cultures in tables I to V, calculated by CONRAD's method (15) in combination with LOVE's table (19) is reported in table V.

The synoptic expressions introduced in the two former papers of this series to replace the long appellations of the different treatments have also been adopted for this paper. Thus, the "plus-iron" cultures will be designated by F and the "minus-iron" by O. Also, the ammonium-nitrogen series will be designated by A-n and the nitrate-nitrogen by N-n.

## Results

### TOTAL NITROGEN

The amounts of total nitrogen varied in different sections of the plants of the various treatments as reported in tables I to IV. These data show that more nitrogen accumulated in the terminal sections of the leaves and in the apical ones of the stem than in the basal or intermediate sections of these organs. Similar results also were obtained in former studies (31, 32). Total nitrogen values were greater in the chlorophyllose sections of the leaves of the A-n than N-n series, also in agreement with other results reported previously (31). The higher values of total nitrogen in the stem and in many of the basal sections of the leaves in the N-n than A-n series resulted possibly from accumulations of nitrate-nitrogen which, in accordance with former studies (31, 32), is not assimilated readily in the non-chlorophyllose tissues of the plant. However, assimilation of nitrate is very rapid in the chlorophyllose tissues of the leaves.

Absorption of nitrogen by the plants, as indicated by the total nitrogen content of tissues, was greater from the F than O cultures. Moreover, the plants of the F cultures in the A-n series contained greater amounts of nitrogen than those in the N-n series. There was, in this connection, good correlation between amounts of total nitrogen and chlorophyll in the leaves (29, 33). The absorption and the subsequent assimilation of nitrate was greater from the F than O cultures in the N-n series which suggests an increased physiological activity in the F cultures, presumably on account of greater chlorophyll contents resulting from the presence of ample amounts of iron. The differences in the amounts of total nitrogen between the F and O cultures in the A-n series were small, and they were possibly induced by the relatively small differences in the physiological activity of these plants on account of the small variation in chlorophyll content.

### TOTAL ORGANIC NITROGEN

Total organic nitrogen, comprising all fractions except the nitrate- and ammonium-nitrogen, was generally higher in the F than O cultures. The differences in organic nitrogen between the F and O cultures were greater in the A-n than in the N-n series as reported in tables I to IV. Organic nitrogen in the A-n series was not consistently greater in the F than O cultures in all sections of the leaves and stem. However, the data in table V show that the differences in the leaves were highly significant between the F and O cultures.

Organic nitrogen values in leaves and roots were inversely proportional, being high in the roots of cultures containing low amounts in the leaves, and vice versa. For example, the organic nitrogen values of the roots were greater in the O than in the F cultures, being  $95.0 \left( \frac{1.288 - 0.662}{0.662} \right) \times 100$  and  $25.7 \left( \frac{1.176 - 0.936}{0.936} \times 100 \right)$  per cent. for the N-n and A-n series, respec-



TABLE I  
FRACTIONS OF INORGANIC, SOLUBLE ORGANIC, AND PROTEINACEOUS-NITROGEN IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR., GROWN IN MINUS-IRON SOLUTION CULTURES SUPPLIED WITH NITRATE-NITROGEN

PLANT SECTIONS	MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE									
	INORGAN.		SOLUBLE ORGANIC				PROTEINACEOUS			TOTAL
	NITRATE	PEPTIDE	AMIDE	MONO-AMINO	BASIC	TOTAL	PROTEOSE AND PEPTONE	PROTEIN	TOTAL	
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Leaves										
Old (B)										
1 + 2 (base)	0.177	0.018	0.046	0.140	0.034	0.238	0.040	0.544	0.584	0.999
3	0.015	0.024	0.088	0.108	0.030	0.250	0.060	0.738	0.698	1.063
4	0.000	0.038	0.058	0.160	0.060	0.316	0.048	0.884	0.932	1.248
5 (tip)	0.000	0.036	0.066	0.180	0.048	0.330	0.052	1.004	1.056	1.388
Mature (C)										
1 (base)	0.364	0.006	0.032	0.184	0.032	0.254	0.018	0.376	0.394	1.012
2	0.318	0.012	0.036	0.136	0.030	0.214	0.028	0.526	0.554	1.086
3	0.029	0.018	0.026	0.108	0.012	0.164	0.036	0.842	0.878	1.071
4	0.009	0.040	0.060	0.172	0.042	0.314	0.046	1.004	1.050	1.373
5 (tip)	0.000	0.048	0.070	0.184	0.068	0.370	0.066	1.164	1.230	1.600
Active (D)										
1 (base)	0.455	0.012	0.044	0.244	0.042	0.342	0.034	0.354	0.388	1.185
2	0.476	0.012	0.022	0.108	0.012	0.154	0.034	0.398	0.432	1.062
3	0.074	0.044	0.048	0.188	0.062	0.342	0.046	0.840	0.886	1.376
4	0.009	0.026	0.024	0.108	0.018	0.176	0.040	1.106	1.146	1.331
5 (tip)	0.000	0.040	0.040	0.128	0.056	0.264	0.060	1.280	1.340	1.604
Young (E)										
1 (base)	0.174	0.102	0.168	0.184	0.022	0.476	0.200	0.170	0.370	1.020
2	0.185	0.016	0.052	0.184	0.030	0.282	0.018	0.482	0.500	0.967
3	0.077	0.036	0.046	0.168	0.064	0.314	0.054	0.684	0.738	1.129
4 (tip)	0.006	0.050	0.048	0.180	0.068	0.346	0.068	1.042	1.110	1.462
Stem										
Base	0.190	0.068	0.094	0.256	0.226	0.644	0.082	0.654	0.736	1.570
Middle	0.222	0.032	0.126	0.428	0.186	0.772	0.078	0.780	0.858	1.852
Apex	0.278	0.074	0.132	0.608	0.134	0.948	0.066	0.826	0.892	2.118
Roots	0.208	0.020	0.052	0.068	0.008	0.148	0.142	0.998	1.140	1.496

\* Organic plus inorganic nitrogen.

TABLE II  
FRACTIONS OF INORGANIC\*, SOLUBLE ORGANIC\* AND PROTEINACEOUS-NITROGEN IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR., GROWN IN PLUS-IRON (5 MG. PER L.) SOLUTION CULTURES SUPPLIED WITH NITRATE-NITROGEN

PLANT SECTIONS	MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE										
	INORGAN.	SOLUBLE ORGANIC					PROTEINACEOUS			TOTAL	
		NITRATE	PEPTIDE	AMIDE	MONO-AMINO	BASIC	TOTAL	PROTEOSE AND PEPTONE	PROTEIN	TOTAL	ORGANIC
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Leaves											
Old (B)											
1 + 2 (base)	0.326	0.022	0.048	0.108	0.024	0.202	0.232	0.420	0.652	0.854	1.180
3	0.029	0.050	0.030	0.132	0.048	0.260	0.418	0.614	1.032	1.292	1.321
4	0.009	0.070	0.038	0.148	0.050	0.306	0.446	0.886	1.332	1.638	1.647
5 (tip)	0.000	0.104	0.052	0.184	0.078	0.418	0.382	1.164	1.546	1.964	1.964
Mature (C)											
1 (base)	0.557	0.030	0.038	0.148	0.026	0.242	0.154	0.120	0.274	0.516	1.073
2	0.535	0.044	0.034	0.160	0.040	0.278	0.268	0.368	0.636	0.914	1.449
3	0.075	0.072	0.034	0.156	0.060	0.322	0.454	0.802	1.256	1.578	1.653
4	0.022	0.082	0.040	0.148	0.074	0.344	0.444	1.000	1.444	1.788	1.810
5 (tip)	0.010	0.108	0.048	0.192	0.086	0.434	0.474	1.196	1.670	2.104	2.114
Active (D)											
1 (base)	0.625	0.062	0.054	0.212	0.038	0.366	0.206	0.192	0.398	0.764	1.389
2	0.521	0.066	0.072	0.164	0.028	0.330	0.194	0.314	0.508	0.838	1.359
3	0.079	0.062	0.040	0.220	0.080	0.402	0.362	0.616	0.978	1.380	1.459
4	0.015	0.112	0.054	0.216	0.158	0.540	0.474	1.220	1.694	2.234	2.249
5 (tip)	0.000	0.138	0.066	0.284	0.180	0.668	0.474	1.580	2.054	2.722	2.722
Young (E)											
1 (base)	0.506	0.136	0.064	0.232	0.048	0.480	0.332	0.386	0.718	1.198	1.704
2	0.268	0.060	0.038	0.148	0.034	0.280	0.230	0.386	0.616	0.896	1.164
3	0.044	0.090	0.036	0.164	0.072	0.362	0.328	0.656	0.984	1.346	1.390
4 (tip)	0.000	0.092	0.046	0.168	0.150	0.456	0.438	1.254	1.692	2.148	2.148
Stem											
Base	0.318	0.158	0.094	0.292	0.092	0.636	0.212	0.574	0.786	1.422	1.740
Middle	0.529	0.270	0.124	0.372	0.116	0.882	0.430	0.506	0.936	1.818	2.347
Apex	0.595	0.360	0.154	0.452	0.120	1.086	0.488	0.582	1.070	2.156	2.751
Roots	0.278	0.026	0.018	0.024	0.018	0.086	0.290	0.286	0.576	0.662	0.940

\* Organic plus inorganic nitrogen.

TABLE III  
FRACTIONS OF SOLUBLE ORGANIC- AND PROTEINACEOUS-NITROGEN IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR.,  
GROWN IN MINUS-IRON SOLUTION CULTURES SUPPLIED WITH AMMONIUM NITROGEN

PLANT SECTIONS	MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE							
	SOLUBLE ORGANIC				PROTEINACEOUS			
	PEPTIDE	AMIDE	MONO-AMINO	BASIC	TOTAL SOLUBLE ORGAN.	PROTEOSE AND PEPTONE	PROTEIN	TOTAL PROTEIN-ACEOUS
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Leaves								
Old (B)								
1 + 2 (base)	0.046	0.278	0.324	0.052	0.700	0.056	0.724	1.480
3	0.064	0.172	0.476	0.238	0.950	0.074	1.172	2.196
4	0.124	0.142	0.480	0.410	1.156	0.100	1.696	2.952
5 (tip)	0.120	0.160	0.460	0.536	1.276	0.112	1.936	3.324
Mature (C)								
1 (base)	0.038	0.324	0.376	0.032	0.770	0.054	0.428	1.252
2	0.046	0.284	0.428	0.058	0.816	0.046	0.712	1.574
3	0.088	0.200	0.472	0.222	0.982	0.074	1.334	2.390
4	0.110	0.178	0.520	0.312	1.120	0.100	1.770	2.990
5 (tip)	0.124	0.192	0.584	0.388	1.288	0.104	2.238	3.730
Active (D)								
1 (base)	0.044	0.246	0.396	0.038	0.724	0.046	0.368	1.138
2	0.036	0.110	0.200	0.040	0.386	0.028	0.550	0.964
3	0.100	0.164	0.420	0.122	0.806	0.048	1.126	1.980
4	0.140	0.098	0.544	0.406	1.188	0.064	1.664	2.916
5 (tip)	0.160	0.206	0.456	0.456	1.278	0.116	2.086	3.480
Young (E)								
1 (base)	0.050	0.140	0.268	0.086	0.544	0.072	0.604	1.220
2	0.046	0.124	0.256	0.064	0.490	0.028	0.642	1.160
3	0.090	0.152	0.344	0.160	0.746	0.046	1.048	1.840
4 + 5 (tip)	0.110	0.164	0.496	0.374	1.144	0.060	1.778	2.982
Stem								
Base	0.054	0.214	0.400	0.146	0.814	0.144	0.878	1.836
Middle	0.062	0.344	0.520	0.152	1.078	0.144	0.728	1.950
Apex	0.078	0.336	0.556	0.174	1.144	0.176	0.774	2.094
Roots	0.032	0.092	0.120	0.022	0.266	0.106	0.804	1.176

tively, whereas those in the combined sections of the leaves and stem were greater in the F than O cultures, being  $30.7 \left( \frac{1.503 - 1.150}{1.150} \times 100 \right)$  and  $5.89 \left( \frac{2.286 - 2.159}{2.159} \times 100 \right)$  per cent. for the N-n and A-n series, respectively.

#### NITRATE NITROGEN

Nitrate nitrogen was more abundant in the F than O cultures of the N-n series as reported in tables I and II. Since both cultures were supplied with equal amounts of nitrate nitrogen, the higher nitrate content of the F cultures resulted from a greater rate of absorption of this ion by these plants, which was confirmed subsequently by certain studies (unpublished) where the nitrate content of nutrient solutions of F and O cultures was determined at the beginning and end of the 2-week period of plant growth. The low nitrate content of the plants of the O cultures in the N-n series resulted from a decreased rate of nitrate absorption which might have been initiated by the depressed rate of growth and metabolic activity on account of the low chlorophyll content of the leaves. The data showing that the weights of the roots were approximately the same in the O and in the F cultures of the N-n series emphasize that the greater content of nitrate in the F cultures was not caused by differences in root surface area but by the differential growth rate and physiological activity as postulated above. The data suggest, moreover, that chlorophyll *per se* did not take direct part in the mechanism of nitrate nitrogen reduction and probably assimilation but that the biochemical and physiological conditions were more propitious for this mechanism in the chlorophyllous than in the non-chlorophyllous regions of the leaves. However, in spite of the low chlorophyll content of the leaves of the O cultures, nitrate nitrogen did not accumulate in the plants, indicating that its reduction and assimilation proceeded satisfactorily.

#### AMMONIUM NITROGEN

The values of ammonium nitrogen were low in the plants of both series and ranged as mentioned before from 0.002 to 0.020 mg. per gram of fresh tissue. Since inorganic nitrogen from ammonium salts is readily assimilated in the tissues of the roots and of other organs (31) the small amounts encountered might have originated in hydrolyzed organic compounds. Recent investigations have shown that this fraction of nitrogen might be derived from glutamic or carbamic acid peptides which form from proteins undergoing hydrolysis, according to MELVILLE (22) and CORWIN and DAMEREL (16).

#### SOLUBLE ORGANIC NITROGEN

The amounts of soluble organic nitrogen, reported in tables I to IV, represent the sum of the amide-, mono-amino-, basic-, and peptide-nitrogen fractions. The proteose-peptone-nitrogen fraction was not included because of its closer relationship to proteinaceous- than to crystalloid-nitrogen.

TABLE IV

PLANT SECTIONS	MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE									
	SOLUBLE ORGANIC					PROTEINACEOUS				TOTAL ORGAN.
	PEPTIDE	AMIDE	MONO-AMINO	BASIC	TOTAL SOLUBLE ORGAN.	PROTEOSE AND PEPTONE	PROTEIN	TOTAL PROTEIN-ACEOUS		
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Leaves										
Old (B)	0.044	0.344	0.360	0.046	0.794	0.110	0.654	0.764	1.558	
1 + $\frac{2}{3}$ (base)	0.058	0.224	0.576	0.128	0.986	0.054	1.462	1.516	2.502	
3	0.066	0.290	0.532	0.310	1.198	0.098	2.052	2.150	3.348	
4	0.090	0.324	0.652	0.430	1.496	0.116	2.264	2.380	3.876	
5 (tip)										
Mature (C)	0.034	0.334	0.300	0.044	0.712	0.118	0.378	0.496	1.208	
1 (base)	0.050	0.274	0.320	0.092	0.736	0.068	0.864	0.932	1.668	
2	0.048	0.166	0.444	0.130	0.788	0.058	1.658	1.716	2.504	
3	0.082	0.174	0.484	0.196	0.936	0.092	2.142	2.234	3.170	
4	0.080	0.170	0.632	0.296	1.178	0.104	2.500	2.604	3.782	
5 (tip)										
Active (D)	0.038	0.268	0.384	0.050	0.740	0.078	0.344	0.422	1.162	
1 (base)	0.022	0.182	0.184	0.042	0.430	0.026	0.606	0.632	1.062	
2	0.050	0.100	0.364	0.122	0.636	0.044	1.446	1.490	2.126	
3	0.092	0.138	0.532	0.232	0.994	0.060	2.100	2.160	3.154	
4	0.092	0.186	0.584	0.350	1.212	0.086	2.476	2.562	3.774	
5 (tip)										
Young (E)	0.098	0.330	0.324	0.070	0.822	0.142	0.330	0.472	1.294	
1 (base)	0.038	0.084	0.236	0.070	0.428	0.030	0.636	0.666	1.094	
2	0.068	0.084	0.368	0.110	0.630	0.034	1.190	1.224	1.854	
3	0.102	0.114	0.540	0.220	0.976	0.076	1.918	1.994	2.970	
4 + 5 (tip)										
Stem										
Base	0.064	0.196	0.376	0.064	0.700	0.174	0.874	1.048	1.748	
Middle	0.072	0.320	0.444	0.092	0.928	0.188	0.790	0.978	1.906	
Apex	0.076	0.410	0.624	0.128	1.238	0.182	0.830	1.012	2.250	
Roots	0.028	0.096	0.124	0.016	0.264	0.052	0.620	0.672	0.936	

Soluble organic nitrogen was higher in the meristematic tissues, namely in the basal-leaf (no. 1) and apical-stem sections, than in the adjacent transitional (no. 2) sections of the D and E leaves. In the chlorophyllose sections (no. 3, 4, and 5) the values increased to higher levels than in the basal (no. 1) sections. The values of soluble organic nitrogen in the tissues were higher in the A-n than in the N-n series, indicating that more ammonium- than nitrate-nitrogen was absorbed and assimilated by the plants. The effects of the F vs. the O cultures on the soluble organic nitrogen content of the tissues differed in the two series. In the N-n series the values of nitrogen were, with few exceptions, higher in the F than in the O cultures. In the A-n series they were only higher in the meristematic tissues; namely, in the basal (no. 1) sections of the active (D) and young (E) leaves and in the apical section of the stem of the F cultures, but in all other sections except in the old (B) leaves they were higher in the O series.

#### AMIDE NITROGEN

Amide nitrogen values reported in tables I to IV and depicted in figure 1, were greater in the A-n than in the N-n series.

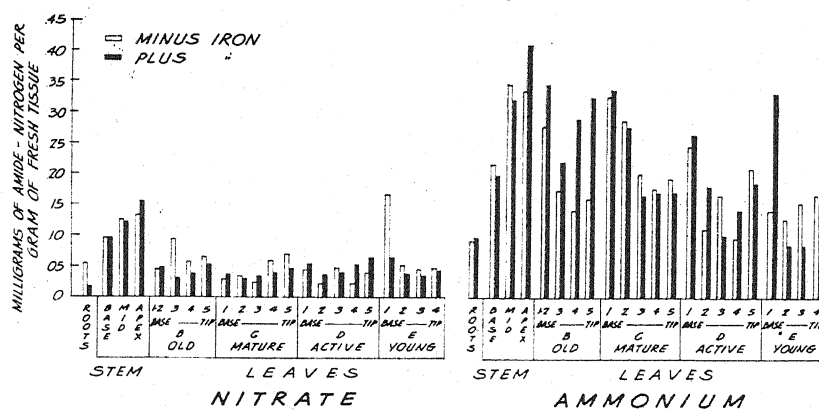


FIGURE 1

FIG. 1. Amide-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

The effects of plus- or minus-iron cultures on the amide nitrogen content of the tissues are confusing. Amide nitrogen values in the basal (no. 1) sections of all groups of leaves, except in the young (E) of the N-n series, were higher for the F than O cultures. The amide nitrogen content of the young (E) leaves, except of the basal section of the A-n series, was higher in the O than F cultures. Uniformly higher values for amide nitrogen occurred in the leaves of the F cultures of the A-n series. In all other groups of leaves amide nitrogen values varied considerably in comparable sections between the F and O cultures.

Amide nitrogen was higher in the apical sections of the stem of the F cultures but lower in the medial and basal sections. This fraction is gen-



erally more abundant in meristematic than in highly differentiated tissues of most plants, and the present findings are in complete agreement with data formerly reported (31) on the distribution of asparagine in *A. comosus*.

#### MONO-AMINO-NITROGEN

Mono-amino-nitrogen, comprising a great portion of alpha-amino-nitrogen, determined but not reported here, and other fractions than those determinable by the Van Slyke method are reported in tables I to IV and depicted in figure 2. This fraction was equally high in the leaves and stem of the A-n series, whereas in the N-n series the stem contained greater amounts than the leaves.

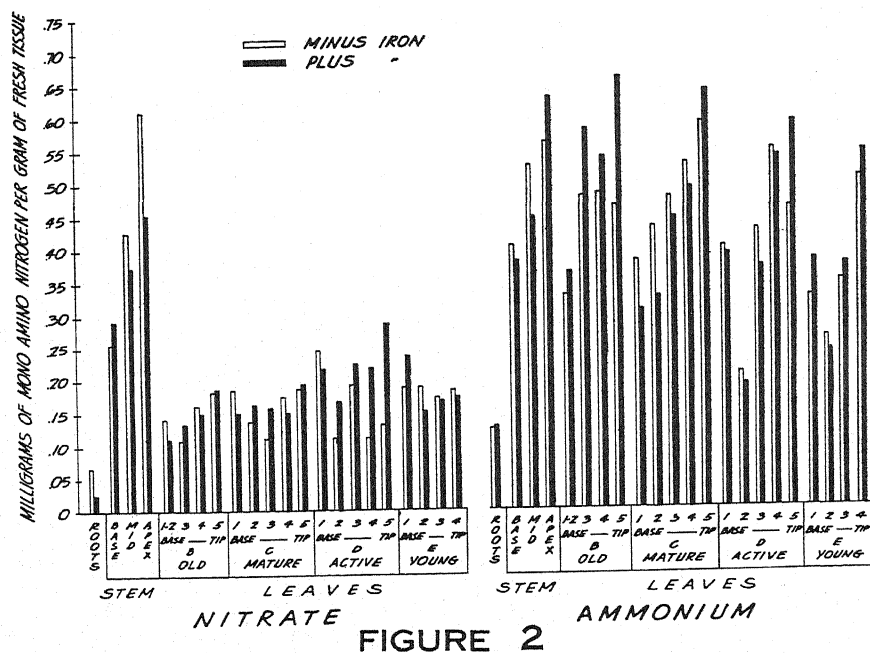


FIG. 2. Mono-amino-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

Mono-amino-nitrogen was high in meristematic tissues such as the basal (no. 1) sections of the active (D) and young (E) leaves and the apex of the stem. Its values increased gradually from the low chlorophyllose (no. 3) to the terminal chlorophyllose (no. 5) sections of the leaves in the active (D) and young (E) groups. In the other groups of leaves the gradient of distribution varied slightly.

The effects of plus- or minus-iron cultures on the mono-amino-nitrogen content of plant sections cannot be clearly defined from the data in figure 6. In the young (E) leaves the basal (no. 1) sections of the F cultures contained higher amounts of mono-amino-nitrogen in both series. In the other sections of these leaves, except the transitional (no. 2), the amounts for the F cul-

tures were high in the A-n series but low in the N-n series. In the active (D) leaves mono-amino-nitrogen was higher in all sections, except in the terminal (no. 5), of the O than F cultures in the A-n series. In the N-n series these values were higher for the F than O cultures except in the basal (no. 1) section. In the mature (C) leaves the values were high for the F cultures in the A-n series but not in the N-n series as shown in figure 6. More mono-amino-nitrogen accumulated in all sections of the stem in the O than in the F cultures except in the apical of the A-n and basal of the N-n series.

#### BASIC NITROGEN

Basic nitrogen, containing some alpha-amino-nitrogen and certain other not well defined fractions, is reported in tables I to IV and depicted in fig-

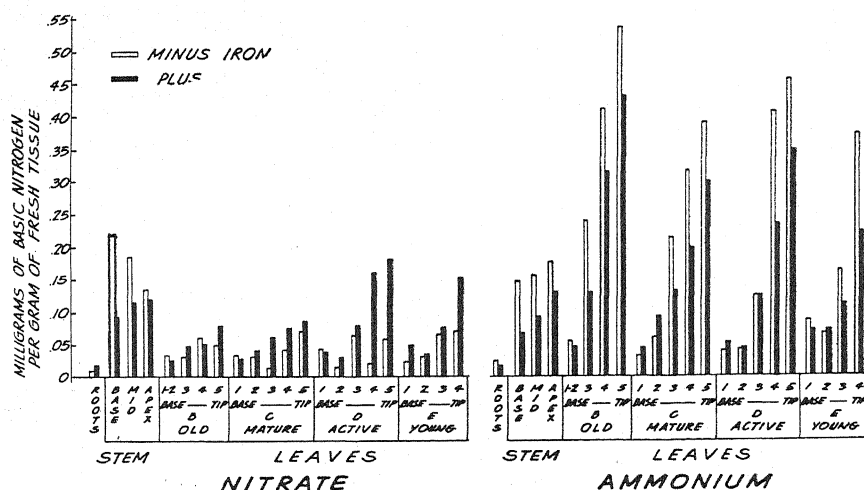


FIGURE 3

FIG. 3. Basic-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

ure 3. The amounts of this fraction were higher in the chlorophyllose sections of the leaves of the A-n than in the N-n series. The differences in the amounts of basic nitrogen in the (no. 1) basal non-chlorophyllose sections of the leaves and in the stem between the A-n and N-n series were small. Basic nitrogen values were generally higher in the O than in the F cultures of the A-n series. However, a few insignificant exceptions were noted. In the N-n series, these values were greater in most leaf sections of the F than O cultures, exceptions being the basal (no. 1) sections of the active (D), mature (C), and old (B) leaves, and the medial chlorophyllose (no. 4) section of the latter group. In the stem these values were reversed, being higher in the O than in the F cultures.

#### PEPTIDE NITROGEN

The amounts of peptide nitrogen, reported in tables I to IV and depicted in figure 4, differed considerably between the F and O cultures of the N-n

series, whereas in the A-n series such differences were small. The differences in the N-n series were from 2 to 8 times greater in the F than O cultures and were statistically significant, as reported in table V. Peptide nitrogen in the A-n series was relatively more abundant in the leaves of the O than of the F cultures but such differences were not highly significant statistically. Fluctuations in the amounts of peptide nitrogen between different plant sections were less pronounced in the F than in the O cultures for both nitrogen series.

#### PROTEOSE AND PEPTONE NITROGEN

These combined fractions of nitrogen, although soluble in the extracting medium, are related more to the proteinaceous- than the crystalloid-nitrogen fractions.

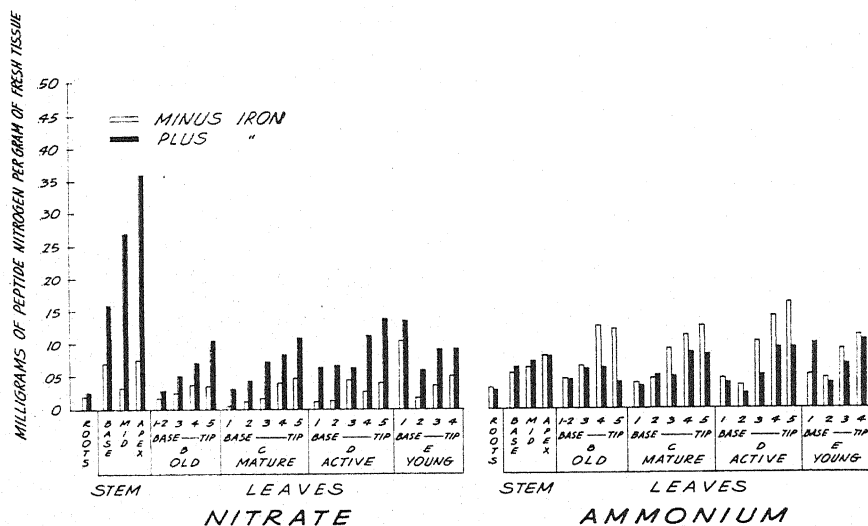


FIGURE 4

Fig. 4. Peptide-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

The amounts of the combined proteose-peptone nitrogen fraction, reported in tables I to IV and depicted in figure 5, were greater in the F than in the O cultures of the N-n series. The difference of the means was statistically significant, as reported in table V. Such differences between the F and O cultures in the A-n series were small and inconsistent and lacked statistical significance. However, in the meristematic tissue of the leaves, namely in the basal (no. 1) section, proteose-peptone nitrogen values were higher in the F cultures, whereas in most other sections (3, 4, and 5) they were higher in the O cultures.

The proteose-peptone nitrogen content of the stem was higher in the F cultures of both series.

## PROTEIN NITROGEN

The amounts of protein nitrogen of the plant tissues, reported in tables I to IV and depicted in figure 6, were affected by the sources of inorganic nitrogen and the amounts of available iron in the nutrient solution. Such amounts were also influenced by the physiological status and the functional specialization of the tissues in the different sections. For example, the plants in the A-n series contained more protein nitrogen than in the N-n series because the rate of ammonium nitrogen absorption from the solution culture and its subsequent assimilation by the tissues was greater than that of nitrate nitrogen, as reported in a former study (31). Also, the amounts of protein nitrogen in the chlorophyllose sections of the A-n and in many of the N-n series of the leaves were greater for the F than for the O cultures.

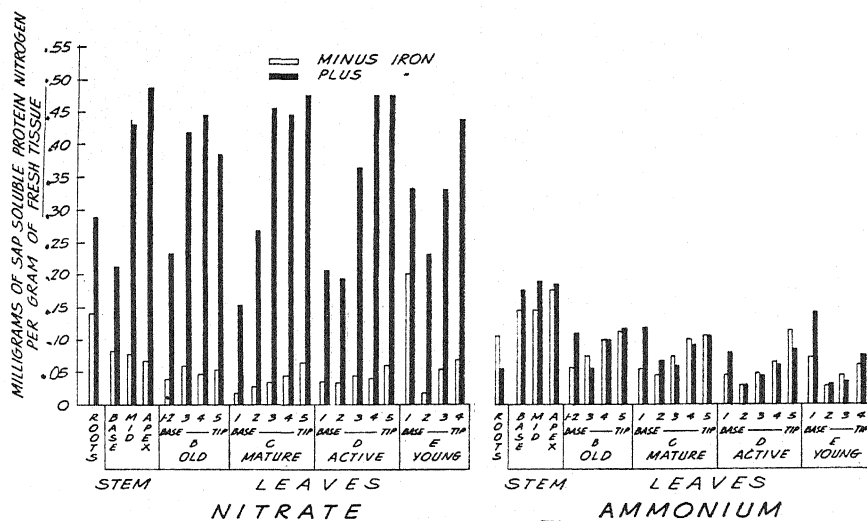


FIGURE 5

FIG. 5. Sap-soluble, inorganic-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

Chlorophyll differences between the F and O cultures in the A-n were small as compared to those between the same cultures in the N-n series. However, the differences in amounts of protein nitrogen of comparable sections between the F and O cultures were greater in the A-n than in the N-n series. These comparisons reveal that certain other factors in addition to the chlorophyll content of the leaves affected the protein content of the tissues. Protein nitrogen values in the stem, being greater for the O than F cultures in the N-n series, suggest that they resulted from concentration effects on account of smaller stem weights in the former (428 gm.) than in the latter cultures (609 gm.) However, the protein nitrogen content of the stems in the A-n series, where stem weights for both cultures were approximately the same, was greater in the F than in the O cultures and behaved in this respect like the leaves.

## Discussion

The data on the effects of iron in the assimilation and in the subsequent metabolic changes of ammonium- or nitrate-nitrogen by *A. comosus* suggest that iron had affected the course of such processes only indirectly. The direct effects of iron can be traced only as far as the formation of chlorophyll. It appears that beyond this point chlorophyll more than iron dominated the course of the various physiological processes. Data presented in the second paper of this series (31) showed that the plants of the O cultures of the N-n series contained smaller quantities of chlorophyll than any of the other cultures. The same plants also contained, with the exception of a few

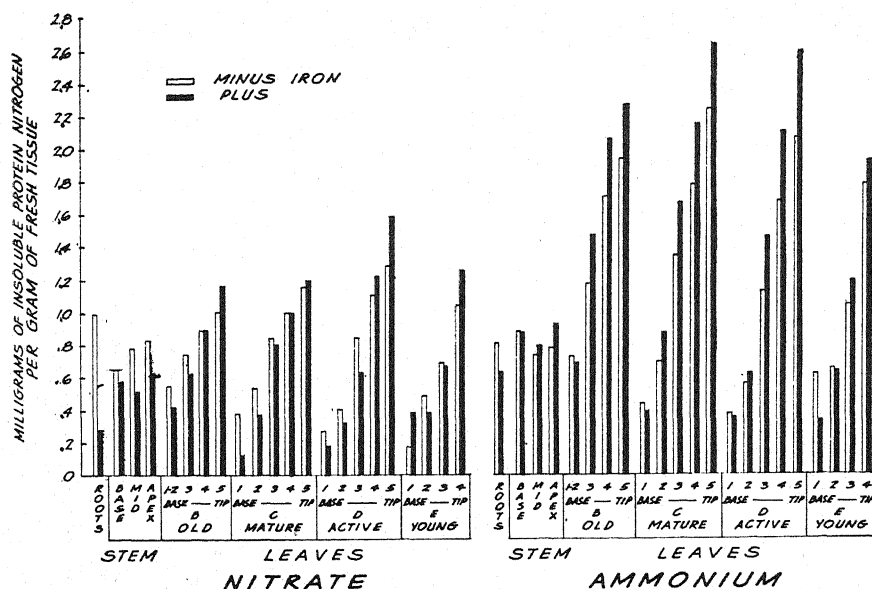


FIGURE 6

FIG. 6. Insoluble-protein-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

sections, smaller amounts of total organic-, nitrate-, soluble organic-, peptide-, and proteose-nitrogen. Differences between the O and F cultures of the N-n series in mono-amino-, basic-, amide-, and protein-nitrogen fractions were not consistent. The greater amounts of total organic- and nitrate-nitrogen in the plants of the F than O cultures of the N-n series should be attributed to a higher rate of absorption and assimilation of nitrate nitrogen by the former plants. The data also suggest that optimum amounts of chlorophyll coupled with favorable conditions for uninterrupted carbohydrate synthesis were essential factors for a high rate of nitrate absorption and assimilation.

The amounts of the various nitrogenous fractions varied between comparable sections of the plants of the F and O cultures in the two series. Also,

the amounts of certain nitrogen fractions differed appreciably in the two series. For instance, the amounts of mono-amino-, basic-, amide-, and protein-nitrogen were greater in the A-n series, whereas those of peptide- and proteose-peptone-nitrogen were greater in the N-n series.

In many leaf and stem sections of the A-n series the amounts of soluble organic nitrogen were greater in the O than in the F cultures, which might indicate that either certain fractions in the soluble organic nitrogen were not converted into proteins as rapidly in the O as in the F cultures because of restricted plant growth or that proteins were hydrolyzed at a greater rate in the O than F cultures.

Examination of the data in the light of recent investigations on nitrogen metabolism suggest a somewhat different course for the assimilation of

TABLE V

CALCULATED STATISTICAL SIGNIFICANCE, BY CONRAD'S FORMULA AND FROM LOVE'S TABLE, OF THE DIFFERENCE BETWEEN THE AMOUNTS OF THE SUBSTANCES LISTED BELOW OF COMPARABLE SECTIONS OF THE LEAVES AND STEM OF PLANTS GROWN EITHER IN "PLUS IRON" OR IN "MINUS IRON" CULTURES AND SUPPLIED EITHER WITH NITRATE OR AMMONIUM SALTS AS SOURCES FOR NITROGEN

SUBSTANCES	NITRATE-N				AMMONIUM-N			
	LEAVES		STEM		LEAVES		STEM	
	SIG- NIFI- CANCE	IN FAVOR OF CUL- TURE	SIG- NIFI- CANCE	IN FAVOR OF CUL- TURE	SIG- NIFI- CANCE	IN FAVOR OF CUL- TURE	SIG- NIFI- CANCE	IN FAVOR OF CUL- TURE
Organic-N .....	9999: 1	+ Fe	55: 1	+ Fe	5000: 1	+ Fe	None	.....
Peptide-N .....	9999: 1	+ Fe	25: 1	+ Fe	30: 1	- Fe	None	.....
Amide-N .....	None	.....	None	.....	None	.....	None	.....
Mono-amino-N .....	None	.....	None	.....	22: 1	+ Fe	None	.....
Basic-N .....	100: 1	+ Fe	None	.....	3000: 1	- Fe	65: 1	- Fe
Proteose-N .....	9999: 1	+ Fe	25: 1	+ Fe	None	.....	None	.....
Protein-N .....	None	.....	25: 1	- Fe	9999: 1	+ Fe	None	.....

ammonium and nitrate nitrogen. SIDERIS, KRAUSS, and YOUNG (30, 31) have shown in *Pandanus veitchii* Hort., and in *Ananas comosus* (L.) Merr., and VICKERY *et al.* (36) in *Nicotiana tabacum* L., that the amounts of amide-N and amino-N were greater when these plants were supplied with ammonium nitrogen but smaller when the source of nitrogen was nitrate. The above mentioned results indicated that the primary products of ammonium assimilation were amide-N and amino-N compounds and suggest that protein synthesis in plants directly absorbing ammonium may be via these primary products. But in the plants absorbing nitrate from solution cultures the small amounts of amide-N and amino-N compounds suggest a slightly different course for the assimilation of nitrate than that of ammonium. BARTON-WRIGHT and MCBAIN (3) claim that in *Solanum tuberosum* L. protein synthesis is direct from nitrate-N via resident-N and probably not via amino acids. The residual-N fraction reported by these authors is comparable to the combined peptide-proteose-peptone fraction reported in these studies.



The presence of proteose-nitrogen in plants has interested many investigators. McKIE (21) claims that, "proteoses are the precursors and immediate decomposition products of the proteins and also that, while proteins are being rapidly synthesized in plants, proteoses diminish and vice versa." That proteins are susceptible to splitting by plant enzymes into disaggregates comparable to proteoses has been shown by BLAGOVESHCHENSKI and SOSSIEDOV (10) and BLAGOVESHCHENSKI and YURGENSON (11) who claim that flour enzymes disaggregate proteins without setting free amino groups from polypeptide chains, indicating that the peptide linkage is not being attacked.

Recent investigations have thrown some light on the mechanism of biological synthesis of amino acids, polypeptides, and proteins in plant and animal tissues. Studies by BRAUNSTEIN and KRITZMAN (12, 13) and COHEN (14) have shown that in plant and animal tissues ammonia is transferred by transaminases, a group of enzymes, from an amino acid to a ketonic acid, with the amino acid being oxidatively deaminated and the ketonic acid reductively aminated. The transfer of ammonia to mono- or di-carboxylic ketonic acids (e.g., pyruvic or oxaloacetic) is directly from dicarboxylic amino acids (glutamic or aspartic) but not from mono-carboxylic amino acids. Certain monocarboxylic acids (lysine, phenylalanine, serine, and leucine) may act as secondary  $\text{NH}_3$  donors, but direct transference of  $\text{NH}_3$  to dicarboxylic ketonic acids is not possible except through the presence of catalytic amounts of dicarboxylic amino acids.

Amines and peptides cannot function as  $\text{NH}_3$  donors. The studies of ADLER *et al.* (1), ALBAUM and COHEN (2), and VITANEN *et al.* (37, 38) are in support of the theory of transamination as postulated above. Other investigations have elucidated certain phases in the mechanism of peptide synthesis in biological systems. Studies *in vitro* by HERBST and SHERWIN (18) in which dl-alanyllalanine was synthesized by transamination from pyruvylalanine and  $\alpha$ -aminophenylacetic acid as the amino donor suggest a scheme for the biological synthesis of peptide chains from non-amino precursors involving amination and acylation. BERGMANN and his colleagues (5, 6) have provided evidence in the synthesis and hydrolysis, by proteinases, of simple peptides from amino acids in the presence of an activator such as cystine. These investigators (4, 8, 9, 10) showed that papain, chymotrypsin, and bromelin were capable of synthesizing amide bonds, such as the synthesis of benzoyl-l-phenylalanyl-l-leucine anilide from benzoyl-l-phenylalanine and l-leucine anilide, indicating recombination of some of the amino acid residues by the synthesis of new peptide bonds in addition to the scission of peptide linkages from proteins. Bromelin is distributed in all organs of *A. comosus* (28), and synthesis of peptide bonds or the scission of peptide linkages from proteins under the conditions of nitrate or ammonium nutrition may affect the peptide and proteose content of the tissues.

High concentrations of amino nitrogen, as shown in the plants of the ammonium series, have been variously explained by different investigators.

PETRIE (23) and PETRIE and WOOD (24) claim that, "a given relation of proteins to amino acids will hold only so long as the proportions between the individual amino acids remain constant; the relation will be different for every change in amino acid proportions." They have based their statement on data which showed that cystine increased less rapidly than other amino acids when the total concentration increased. LUGG and WELLER (19) have also reported a comparable condition where protein synthesis was limited by the low amounts of methionine in the seed.

In this study, the higher concentrations of amide-, amino-, and protein-nitrogen in the ammonium than in the nitrate series may be explained by the greater reactivity of ammonium with dicarboxylic ketonic acids, assuming that these substances are present in the tissues, to form either glutamic or aspartic acids or their corresponding amide fractions as well as other amino acids. The biochemical conditions which are required for the reduction of nitrate and its conversion to organic compounds which, according to our information to date, takes place in the chlorophyllose tissues of the leaves, may retard the rate of synthesis of amide- or amino-nitrogen fractions and thus explain the low amounts of amide- or amino-nitrogen fractions. The physiological mechanism for the reduction of nitrates in the tissues of *A. comosus* is unknown. However, one or more activators which cause the conversion of nitrates into some organic nitrogen fractions, comparable to cystine in the studies of BERGMANN and his colleagues, may be present in the chlorophyllose and lacking in the non-chlorophyllose tissues of the leaves. Such fractions if identical with the amide- and amino-nitrogen in the plants of the ammonium series must have polymerized immediately to such complex compounds as polypeptides.

STEWART and PRESTON (34) have stressed oxygen tension as playing an important rôle in protein synthesis and salt accumulation on the basis of studies on the aeration vs. non-aeration of potato discs suspended in nutrient solutions. In our study the plants of both ammonium and nitrate series were aerated equally. However, molecular oxygen released from reduced  $\text{NO}_3$  molecules might have increased the intracellular tension of oxygen which may act as a catalyst in the polymerization of amide- or amino-nitrogen to polypeptides and proteins.

The data definitely indicate that high amounts of amide- and amino-nitrogen in tissues of *A. comosus* were associated with a source of ammonium in the solution culture while nitrate-, peptide-, or proteose-peptone-nitrogen with one of nitrates. However, different amounts of other nutrient elements or conditions of light and temperature may change materially the amounts of such nitrogen fractions in the tissues by their effects on the carbohydrate reserves and rate of mineral absorption which in turn influence the nitrogen assimilating capacity of plants.

### Summary

1. The plants grown in the ammonium series contained greater amounts of total nitrogen than those in the nitrate nitrogen series. The leaves and

stem but not the roots of the plants of the plus-iron cultures in the ammonium-nitrogen series contained slightly greater amounts of total nitrogen than of the minus-iron cultures. The differences in total nitrogen between the plus- and minus-iron cultures were greater in the nitrate- than ammonium-nitrogen series, and they were in favor of the former cultures.

2. The amide- and amino-nitrogen content of the tissues was greater in the ammonium- than in the nitrate-nitrogen series. In the former series the plus-iron cultures contained more amide- and amino-nitrogen than the minus-iron cultures. In the nitrate-nitrogen series, the plus-iron cultures contained more peptide- and proteose-peptone-nitrogen than the minus-iron cultures. These results indicate that the primary products of ammonium assimilation were amide and amino nitrogen compounds and suggest that protein synthesis in plants supplied with ammonium nitrogen was via these compounds. In the plants supplied with nitrate, protein synthesis from inorganic nitrogen compounds was apparently different than with ammonium.

3. Statistically significant differences were obtained in the amounts of organic-N, peptide-N, basic-N and proteose-peptone-N between the plus- and minus-iron cultures of the nitrate-nitrogen series which were in favor of the plus-iron cultures, whereas in the ammonium-nitrogen series such differences were obtained in the organic-N and protein-N fractions. In the ammonium-nitrogen series, basic nitrogen was the only fraction highly significant in favor of the minus-iron cultures.

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## TRANSPIRATION OF PINE SEEDLINGS AS INFLUENCED BY FOLIAGE COATINGS

HUBERT MARSHALL AND T. E. MAKI

(WITH ONE FIGURE)

Widespread losses frequently occur in conifer plantations during the first weeks after the young trees are set in the field. This early mortality is largely attributable to "physiological drought" resulting from continued transpiration and the concomitant failure of the recently disturbed root systems to make up the daily water deficit. Under such conditions permanent wilting occurs, and death soon follows.

In attempting to reduce this mortality, methods have been sought to encourage the early establishment of the root system and to reduce the excess of transpiration over absorption during the establishment period. This latter approach to the problem has been used with gratifying success by nurserymen who frequently top-prune hardwoods as a means of reducing transpiration and of increasing survival of transplanted stock. Reducing the transpiring surface of conifers by this method has not proved feasible, but it is possible that coating the foliage with various waxes, fats, and oils might produce an equally effective reduction in water loss. This has been attempted but with variable results both in terms of water loss and field survival; to date the practice has not received widespread acceptance.

The literature contains but slight mention of the degree to which transpiration of coniferous seedlings is reduced by foliage coatings; unfortunately the information available has generally been obtained by the use of coatings which, under some conditions at least, have proved of little value for increasing resistance to drought. EMERSON and HILDRETH (1), for example, found that corn oil and several other substances reduced water loss from dormant *Pinus austriaca* seedlings as much as 88.4 per cent. and caused no foliage injury under conditions prevailing in Wyoming. No actual test of drought resistance was reported. However, in tests at Beltsville, Maryland (2), under both field and artificial drought conditions, corn oil caused injury to the foliage of a number of coniferous species and did not materially increase resistance to drought. In similar tests, SHIRLEY and MEULI (4) studied the effects of 1 rubber and 3 wax coatings on two-year-old seedlings of white spruce. Two of the coatings materially reduced transpiration, but in extensive field trials with several species none of the substances increased survival.

A number of experiments (2) conducted during the past several years, both in the field and under conditions of artificial drought, have shown that foliage coatings of emulsified lanolin and a commercial paraffin wax increase first year survival about 10 per cent. Under more specific conditions, such as late fall planting, foliage coatings of these substances may produce even greater benefits. The purpose of this report is to present data showing the



reductions in transpiration resulting from the use of these two coatings under conditions of atmospheric drought. Incidental observations on the relation of transpiration to root volume for several species also are presented.

### Materials and methods

The species used in this study were 2-0 loblolly pine (*Pinus taeda*), 5-0 red pine (*Pinus resinosa*),<sup>1</sup> and 3-0 white pine (*Pinus strobus*), lifted from a local nursery in July, 1944. Thirty uniform seedlings of each species were selected, weighed individually, and divided into three groups of roughly equal weight for treatment with foliage coatings. The following three treatments were used:

1. Control (untreated).
2. Lanolin emulsion at a concentration of 100 grams lanolin (anhydrous Adeps Lanae) per liter of water.
3. Dowax,<sup>2</sup> one part prepared emulsion to three parts water.

The lanolin was emulsified with 10 grams of monoethanolamine stearate per liter of water. The commercial wax required only mixing with three times its weight of water. The seedlings were treated by immersion of the foliage in the proper substance for a few moments followed by a gentle shaking to remove excess coating material.

After treatment the seedlings were potted in no. 2 tin cans measuring  $2\frac{1}{2}$  inches in diameter and  $3\frac{1}{2}$  inches in depth. The potting medium was a fine sand mixed in bulk to a moisture content of 18.2 per cent. based on dry weight. This moisture content was at the field capacity of the sand and was only slightly reduced during the course of the experiment. Water loss was determined daily by weighing the cans on a small trip balance sensitive to 0.1 gram. To prevent loss of water by evaporation from the surface of the sand, the cans were effectively sealed with a half and half mixture of paraffin and beeswax.

During the course of the experiment, the seedlings were exposed to the environmental conditions of the drought machine described by SHIRLEY and MEULI (5). Briefly, this machine consists of a revolving circular table six feet in diameter, enclosed in an insulated metal drum four feet in height. The seedlings rest on the revolving table and are exposed to a thermostatically controlled temperature of 96° F., constant light intensity of about 200 foot-candles from two incandescent bulbs, and a wind velocity of several miles per hour generated by two fans attached to the inner sides of the metal drum. The revolving table is driven by an electric motor and has a velocity at the periphery of 1.4 miles per hour. Humidity in the present test was approximately 38 per cent.

<sup>1</sup> The 5-0 red pine, while not ordinarily used in forest plantings, was the only size class available and provided a satisfactory test object for the purposes of this study.

<sup>2</sup> The commercial wax used in this study is the proprietary product, Dowax, containing a paraffin wax, bentonite, an emulsifying agent, and possibly other substances. The exact composition is unknown to the writers.

At the conclusion of the 5-day exposure period, root volumes of all seedlings were measured to the nearest 0.1 ml. by water displacement in a burette. This information was obtained in order to correlate root volume with transpiration.

### Results and discussion

#### FOUR-DAY WATER LOSS

Despite the existence of ample moisture in the sand, several control seedlings showed some slight evidence of drought stress at the end of the fourth day of exposure. This "physiological drought" is presumably similar to that of seedlings showing drought stress shortly after planting in moist soils in the field. Since the data collected beyond this time were not representative of vigorous seedlings, the results for the first four days are chosen as representing the effects of foliage coatings in the period immediately after planting. The data are shown in table I.

TABLE I

WATER LOSS FROM GROUPS OF 10 SEEDLINGS EXPOSED FOR 4 DAYS TO  
ARTIFICIAL ATMOSPHERIC DROUGHT

SPECIES	TREATMENT	MEAN FRESH WEIGHT OF SEEDLINGS	WATER LOSS IN 4 DAYS		
			ACTUAL MEAN	IN RELATION TO FRESH WEIGHT	IN RELATION TO CONTROLS
		<i>gm.</i>	<i>gm.</i>	<i>%</i>	<i>%</i>
White pine	Control	12.33	27.30	221.41	100.00
	Lanolin	12.60	10.52	83.49	37.71
	Com'l. wax	12.75	10.03	78.67	35.53
Red pine	Control	10.83	16.45	151.89	100.00
	Lanolin	11.01	7.10	64.49	42.46
	Com'l. wax	11.39	5.41	47.50	31.27
Loblolly pine	Control	5.77	9.33	161.70	100.00
	Lanolin	6.01	4.64	77.20	47.74
	Com'l. wax	5.85	3.39	57.95	35.84

Differences in fresh weight within species were not found to be significant when tested by SNEDECOR's analysis of variance method (6). The column showing water loss as percentage of fresh weight provides a comparison of treatment differences in which water losses for all three species are established on the same relative basis. The final column provides the better comparison between controls and the two foliage coatings and shows that treated plants on the average transpired less than 40 per cent. as much as controls. Such a reduction in transpiration presumably is responsible for the increased resistance to drought shown by treated seedlings planted under many conditions in the field.

The differences between control and treated seedlings (table I) were tested by the analysis of variance method and were found to be highly significant. The differences between seedlings treated with lanolin and com-

mercial wax likewise were tested, but highly significant differences were found for only red pine and loblolly pine. Although this latter test gives an estimate of the differences necessary for significance, it should not imply the superiority of any particular coating, since concentrations were arbi-

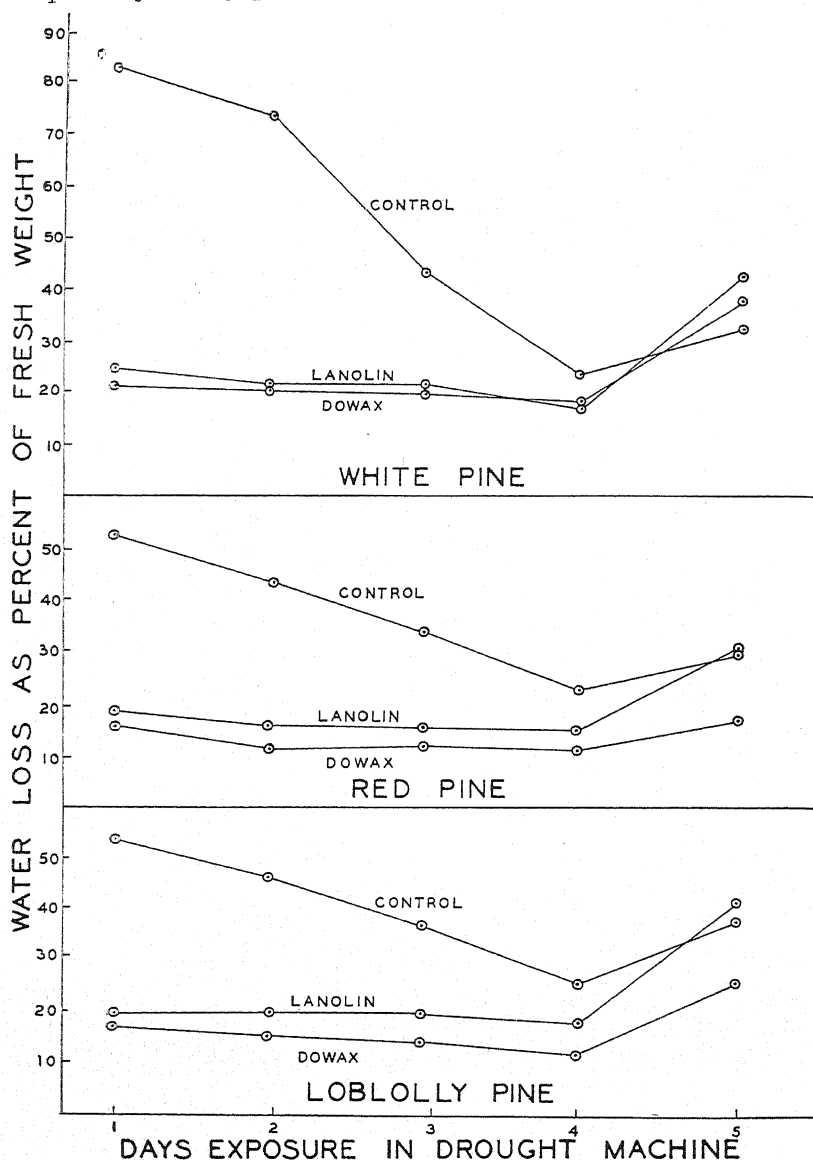


FIG. 1. Water loss of groups of 10 pine seedlings exposed to artificial drought.

trarily selected, and the results would be expected to vary with changes in concentration.

The first four days of exposure provided the basis for the results presented above. On the fifth and final day of exposure, an extra set of bulbs was accidentally illuminated, resulting in a temperature rise from 96° to

110° F. and a drop in the relative humidity from 38 to 21 per cent. At the end of the fifth day all white-pine controls, eight of the ten red-pine controls, and three of the ten loblolly-pine controls showed severe drought injury, and it was apparent that most of the injured seedlings were beyond the point of recovery. Among coated seedlings only three scattered individuals showed a comparable degree of injury, thus providing an indication of the degree of protection afforded by the coatings under drought machine conditions. Samples of sand taken from the cans at the termination of the exposure period showed that all seedlings still had an adequate supply of moisture.

The course of transpiration during the entire 5-day period of exposure is shown graphically in figure 1. Although not proved experimentally, figure 1 indicates that most coated seedlings absorbed sufficient moisture to maintain a uniform daily water loss as modified by the coatings and, either by absorption or the loss of moisture reserves, responded to the drastic conditions of the fifth day of exposure with materially increased transpiration. Control plants, on the other hand, with an initial rate of water loss some three times greater than that of coated plants, were unable to maintain that rate for even a full day. Despite adequate moisture in the sand, the transpiration rate continued to fall, indicating, presumably, a progressive reduction in both moisture reserves and the rate of water absorption. The rise in water loss on the fifth day was not as large as that for coated seedlings, despite the fact that the original rate of water loss was far greater. Thus, the high rate of transpiration resulting from the absence of foliage coatings so exceeded the rate of absorption that most control seedlings were injured beyond the point of recovery by the end of the fifth day of exposure.

#### WATER LOSS IN RELATION TO SEEDLING SIZE

The data in table I are based on mean values, hence they do not provide information on water loss as related to variations in seedling size within each of the three species. To gain information on this relationship, regression equations of the type formula  $Y = a + bX$  were calculated in which  $Y$  represents water loss in grams for the four-day period,  $X$  represents fresh weight of seedlings in grams, and  $a$  represents the  $Y$ -intercept.

The equations are as follows:

##### Control series.

White pine (Range, 7-19 gm.) .....	$Y = 0.523X + 20.85$
Red pine (Range, 6-18 gm.) .....	$Y = 0.957X + 6.09^*$
Loblolly pine (Range, 4-11 gm.) .....	$Y = 0.326X + 7.45$

##### Lanolin series.

White pine (Range, 6-19 gm.) .....	$Y = 0.734X - 1.27^{**}$
Red pine (Range, 6-15 gm.) .....	$Y = 0.939X - 3.24^{**}$
Loblolly pine (Range, 4-9 gm.) .....	$Y = 0.893X - 0.73^*$

##### Commercial wax series.

White pine (Range, 7-20 gm.) .....	$Y = 0.884X - 1.24^{**}$
Red pine (Range, 6-18 gm.) .....	$Y = 0.398X + 0.88^{**}$
Loblolly pine (Range, 3-11 gm.) .....	$Y = 0.525X + 0.32^{**}$

The regression coefficients of the equations marked with single or double asterisks are significant at the 0.05 or 0.01 levels, respectively. Although each equation is based on data from only ten seedlings, more than half of the coefficients are highly significant, thereby reflecting the close association of water loss to seedling weight, a relationship which was demonstrated to be linear within the ranges of the present data. In this regard, it is of interest to note that the commercial wax, and to a lesser extent lanolin, reduced random variation in water loss between individual seedlings sufficiently to afford a statistical verification of the relationship in every instance of the treated series.

#### SPECIES AND ROOT-VOLUME RELATIONSHIPS

The data in table I provide an example of the sizable differences in water loss which occur between closely related species growing in the same environment. Little is known regarding the basis for such differences, but it is possible they are associated with morphological factors such as root volume. To test this possibility, root volumes were determined and are compared with water loss in table II.

TABLE II

WATER LOSS\* DURING 4-DAY DROUGHT EXPOSURE AND ROOT VOLUMES† OF RED AND LOBLOLLY PINES EXPRESSED IN TERMS OF THOSE OF WHITE PINE

SPECIES	RELATIVE WATER LOSS BY TREATMENTS				RELATIVE ROOT VOLUMES BY TREATMENTS			
	CON- TROL	LANO- LIN	COM 'L. WAX	SPECIES MEAN	CON- TROL	LANO- LIN	COM 'L. WAX	SPECIES MEAN
	%	%	%	%	%	%	%	%
White pine .....	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Red pine .....	68.6	77.2	60.4	68.7	81.4	66.8	58.5	68.9
Loblolly pine .....	73.0	92.5	73.7	79.7	93.1	80.5	80.1	84.6

\* Calculated from water loss per gram fresh weight.

† Calculated from root volume in milliliters per gram fresh weight.

Inspection of the table reveals a similarity between the four-day water loss and root volume. White pine, with the largest water loss, likewise had the largest root volume per unit fresh weight; red pine, with the smallest relative water loss, had the smallest root volume. Not only are these species differences consistent for all three treatments, but the mean values are remarkably similar. Although not proved experimentally, the possibility is suggested that, under the conditions of this experiment, transpiration may be largely a function either of root volume or of some associated factor.

These observations were largely a side issue to the main purpose of the experiment and verification of the water-loss-root-volume relationship under other conditions and with other species has not been made. It is understood that there may not be a close association between root volume and transpiration when species having unlike root systems are compared. Between species having similar root systems, however, the relationship appears valid inasmuch as volume, under these circumstances, is closely correlated with

surface area. The present observations, for example, are of importance in studies such as that of SCHOPMEYER (3), where an attempt was made to relate the drought resistance of several species of conifers to their rates of transpiration. Similar studies might profitably consider the relation of transpiration to root volume before conclusions on the relation of transpiration to drought resistance are formulated.

### Summary

The water loss of white pine, red pine, and loblolly pine seedlings was determined gravimetrically during exposure to environmental conditions of high temperature and low humidity. With ample moisture in the potting medium, the following results were obtained:

1. Seedlings top-dipped in emulsions of lanolin or a commercial paraffin wax transpired during four days of exposure less than 40 per cent. as much as untreated seedlings.

2. Untreated seedlings failed to respond to a substantial rise in temperature on the fifth day of exposure with as large an increase in water loss as did coated seedlings. Although not demonstrated experimentally, this response is presumed to result from a severe moisture deficit created in untreated seedlings during the first four days of exposure.

3. On the basis of water loss per unit fresh weight, red and loblolly pines transpired respectively only 68.7 and 79.7 per cent. as much as white pine. The calculation of root volumes per unit fresh weight showed a closely similar relationship.

4. Regression equations demonstrated that water loss varies directly as the fresh weight of the seedling within the range of the present data.

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AN ELECTROMETRIC METHOD FOR DEFINING THE  
AREA OF BARK AFFECTED BY TAPPING  
*HEVEA BRASILIENSIS*

DAVID G. WHITE

(WITH TWO FIGURES)

The principal source of natural rubber is the Para rubber tree [*Hevea brasiliensis* (H.B.K.) Muell. Arg.]. Even after several decades of plantation practice no satisfactory method has been developed for measuring the area of bark affected by tapping. Such measurements are important because modern tapping systems sometimes utilize two cuts on the same tree, and the cuts frequently interfere reciprocally (5, 6, 7, 12). Thus, the separation of panels by an arbitrary distance often results in yields which do not meet expectations, and, in many instances, a physiological disorder called "Brown Bast" develops. This diseased condition is associated with over-tapping (10, 11) and may be caused by the two tapping cuts being too near each other. In parts of some plantations over 80 per cent. of the trees are out-of-tapping because of Brown Bast. Incidence of this magnitude could be avoided if the area of bark influenced by tapping were reasonably defined, and the two cuts spaced accordingly. The purpose of this paper is to explain a method which fulfills the requisites of such measurements. However, the limited number of trees available for the experiment described herein restricts further study at this station. Field applications of this electrometric method would need to be based upon results with select clonal trees found on modern plantations.

Previous methods of measurement

The problem is of sufficient importance that numerous attempts have been made to devise a method to measure the area of bark affected by tapping. Briefly, some of these methods have been: The use of interrupting cuts of various lengths and distances below the tapping cut in such a way that the yield is decreased by cuts made within the border of the "drained" area (2); the tapping of trees which produced a colored or oxidizable latex in a known area of bark below the tapping cut and white latex in the area near the cut (14); mathematical estimations of the hypothetical area of bark concerned for the daily regeneration of latex during a rest period (1); and calculations based upon the volume of latex expressed from a known area of removed bark (4). Each method has had at least one key fallacy, such as measuring only the hypothetical area of bark through which latex moved before it exuded. Since the vessels are never empty there is, of course, movement of latex in an area of bark greater than that merely "drained." According to these methods latex moves to the tapping cut over a distance ranging from 1 to 3.5 meters. The consensus of opinion has been that a distance of

1 meter is sufficient separation for two cuts. However, this arbitrary distance does not allow for differences between high- and low-yielding trees. As a result, high-yielding trees have suffered the most from having cuts too near each other.

#### An electrometric method

The diffusion pressure gradient in the bark below a cut is known to be radically affected by tapping (2, 4, 14). It appears logical that measurement of the distance below the tapping cut over which this gradient occurs



FIG. 1. The Bouyoucos Bridge used in determining variations in resistance of the bark tissues caused by tapping *Hevea brasiliensis*. Hypodermic needles were employed as terminals inserted in the bark (arrows). The usual type of tapping knife is also shown.

also would define the area of bark influenced by tapping. The measurement of the variations in diffusion pressures of the latex and other cell contents can be performed electrically. A method used in this study was to measure the resistance to an electric current offered by the bark tissues and cell contents, including the latex, at intervals below the tapping cut.

Satisfactory terminals were made from discarded hypodermic needles which were shortened to within  $\frac{1}{2}$  inch of the shoulders. The ends were

squared and sharpened. The needles were then inserted firmly in the bark at right angles until woody tissues were encountered. Two terminals were placed exactly 1 inch apart horizontally, and pairs were spaced 6 inches apart vertically (fig. 1). Resistance of the bark tissues to a relatively small electric current was measured in ohms by means of the Bouyoucos Bridge (3). Six-foot, radio-wire leads with spring clamps attached on the terminals permitted rapid operation.

Several virgin seedling trees of *Hevea brasiliensis* 20 to 25 years of age were available for experimentation. Unfortunately none of these trees are equal to high-yielding clonal selections, but, nevertheless, the results are applicable insofar as the method is concerned. After preliminary tests with a few trees, the best one was chosen for demonstrating the method. A half-circumference cut was marked to start 50 inches from the ground. Pairs of terminals were inserted beneath the center of the proposed tapping cut and also on the opposite side at the same heights. Several days elapsed before measurements were started so that wound effects, if any, might become constant. However, later experience indicated that the resistance between terminals became relatively constant within a few hours after the insertion of the terminals.

The first electrometric measurements were made without having tapped the tree in order to determine the usual fluctuations which might be expected to occur. These preliminary tests, made while the trees were in leaf, demonstrated diurnal variations in resistances of the bark tissues to an electric current. These variations often coincided with the usual daily transpiration curves of leafy plants (8, 9). Further investigations with tomato plants indicated the possibility of refining this method as an indirect measurement of transpiration. However, in the present study, differences caused by transpiration of the rubber tree were negligible in comparison with those caused by tapping.

### Results

Electrometric measurements were made at intervals throughout the day for 2 days on the tree shown in figure 1 before the tapping cut was opened. The daily variation in resistance from the initial resistance offered by the bark between individual pairs of terminals was considered an important criterion. In other words, differences between sets of terminals were of no importance as long as they were within a reasonable range of several thousand ohms at the beginning. These beginning differences among sets of terminals located at 6-inch intervals were probably caused by variations in bark structure, the depth to which the terminals penetrated, and the amount of bark separating individual pairs. In any case the electric current followed the course of least resistance between terminals.

The resistances between terminals both beneath the proposed tapping cut and on the opposite side of the trunk varied throughout the day as much as 3,000 to 4,000 ohms. In all instances of daily variation before the tapping cut had been opened there was a decrease in resistance and in no case an

increase. This variation throughout the day is partially explainable on the basis of differences in diffusion pressures caused by translocation (13). *Hevea brasiliensis* is deciduous, and the tree had just begun to form new leaves. However, the precise cause (or causes) of the daily fluctuations was not studied.

Radical changes in resistance brought about by tapping, as shown later, occurred within the first half hour after tapping. Therefore, the variation in resistances which might occur ordinarily during this period was important. During the 2 days prior to opening the tapping cut no variation in resistance more than 1,000 ohms occurred within any half-hour period. These relatively small variations were invariably evident as decreases in resistance. An average variation per pair of terminals amounted to about 250 ohms, although the Bouyoucos Bridge was not accurate to less than 500 ohms at the range employed. In all cases these variations can be considered relatively minute and practically within the margin of error of the instrument.

TABLE I

RESISTANCE OFFERED TO AN ELECTRIC CURRENT BY BARK TISSUES BEFORE AND AFTER TAPPING *Hevea brasiliensis*

DISTANCE OF TERMINALS BELOW TAPPING CUT	PRE-TAPPING RESISTANCE	PERIOD AFTER TAPPING IN MINUTES					
		1-2	5-7	9-12	14-19	21-24	25-28
<i>inches</i>	<i>ohms</i>	<i>ohms</i>	<i>ohms</i>	<i>ohms</i>	<i>ohms</i>	<i>ohms</i>	<i>ohms</i>
6	18,500	21,100	22,000	23,000	21,500	20,000	20,000
12	22,000	24,500	25,000	24,000	23,000	21,500	21,000
18	13,000	13,000	13,500	13,000	12,500	12,000	12,000
24	14,000	14,000	14,000	14,000	13,500	13,000	13,000

On the third day the tapping cut was opened to within 1 mm. of the woody tissues. Tapping was done daily thereafter between 7:30 and 8:00 A.M. Measurements made the next 3 or 4 days cannot be considered typical because this period in tapping is required before the tree yields a more-or-less constant amount of latex. Even so, differences in resistances during this period were found comparable to later measurements. Probably the reason early measurements were comparable with those made after tapping for several days was because of the relatively low yields of latex (8 to 12 ml.). Exudation of latex generally ceased within 15 to 20 minutes.

The data for the first four pairs of terminals below the tapping cut on the fifth day of tapping are presented in table I as typical results of the electric measurements. These data are arranged in groups according to the period of time which had passed after tapping. The tapping operation itself only required 15 to 20 seconds. Variations from the pre-tapping resistance offered between each pair of terminals are plotted in figure 2. Variations in resistances on the opposite side of the trunk were found to be so small that no definite effect of tapping was detectable, and a similar lack of variation

was evident in resistances between terminals placed 30, 36, and 42 inches below the tapping cut. Therefore, for the sake of simplicity, data of these terminals are not included.

### Discussion

The data in table I and figure 2 show that the resistances of the bark tissues increased after tapping in the immediate area beneath the tapping cut. Since the usual variation in resistances was always a decrease on trees not tapped, the increase of resistance in the immediate area of the tapping cut was concluded to have been caused by tapping. These data also show that the effect of tapping was progressively less at 6-inch intervals below the cut. In fact, no definite effect was measurable 24 inches below the cut, and there was only a slight effect 18 inches below the cut. Therefore, tapping

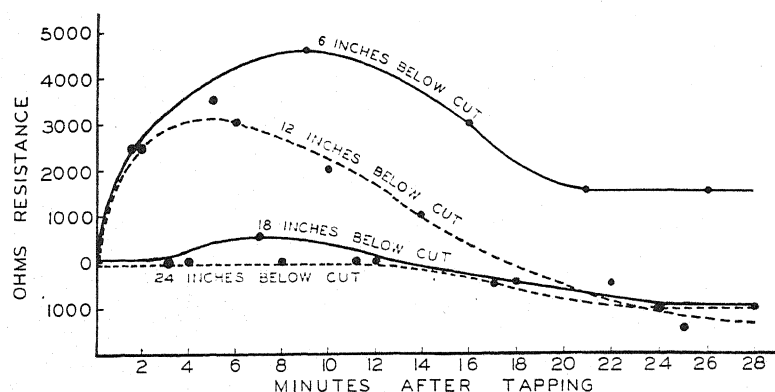


FIG. 2. Variations from the initial resistance to an electric current of bark tissue after tapping *Hevea brasiliensis*. The zero level of the ordinate represents the initial resistance before tapping.

this particular panel was concluded to have affected the bark tissues to a distance of 18 to 24 inches below the cut and to have had no influence on the opposite side of the tree. It is again emphasized that the yields of this seedling tree were relatively low. Thus, these specific results are not applicable to selected clonal trees which often average 75 ml. or more of latex per tapping by the same system. Certainly, the amount of bark influenced by tapping a high-yielding tree would be greater than the amount affected on the seedling tree described here.

A hypothetical explanation of these results is based upon the factors which are associated with the movement of latex. It is probable that the exudation of latex and consequent loss of water caused an increased diffusion pressure in the remaining cell contents to such an extent that resistance was increased. The fact that the effects of tapping were progressively less at 6-inch intervals below the tapping cut is conceivable from a physical standpoint. This method appears to be more exact for defining the area of bark influenced by tapping than previous methods because it measures the vertical declination of tapping effects.

It was further concluded that two cuts in echelon might be safely separated by a distance of 24 inches on the seedling tree studied. The chances of one cut, particularly the high panel, having an area of bark affected by tapping which would overlap that of the lower cut is remote. Thus Brown Bast caused by two cuts being placed too near each other would be avoided. In a few cases Brown Bast is evident from the time of opening the cut. In these instances the inherent characteristics of the tree and not over-tapping are fundamental causes. However, the same distance of separation of the cuts would not apply to high-yielding trees and determinations with them need to be done during periods of their greatest yields. In addition, the lateral areas of bark affected by tapping as well as the distance above the tapping cut are worth further investigation.

### Summary

1. The importance of defining the area of bark influenced by tapping *Hevea brasiliensis* is discussed.
2. An electrometric method is described for measuring the area of bark below a tapping cut which is influenced by tapping.
3. The effects of tapping a low-yielding seedling tree with a half-circumference cut were found measurable to a distance between 18 and 24 inches below the tapping cut.
4. Similar measurements need to be done for high-yielding clonal trees during the periods of their greatest production.

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## BRIEF PAPERS

### ENTRANCE OF WATER INTO SUBERIZED ROOTS OF TREES

RUTH M. ADDOMS

Results of several investigators (1, 2, 3, 4) suggest that the absorbing area of root systems may not be restricted to young roots and the young terminal portions of older roots, but that older suberized portions may account for a considerable amount of the water absorbed. By means of potometers HAYWARD, BLAIR, and SKALING (2) demonstrated an appreciable amount of absorption by suberized roots of sour orange. KRAMER (3) has made a comparative study of rate of absorption by suberized roots of several different species. The present experiment was undertaken to determine the avenues of entrance of water into suberized roots.

#### Material and methods

Suberized roots were immersed in a dye solution and subjected to vacuum; after this treatment they were studied microscopically. The dye that proved most useful was azosulfamide ("Neoprontosil") in a concentration of 1:1000 in water. Treatments lasted 30 minutes; little or no change was noted as a result of longer treatment. Vacuum was obtained by the use of a filter pump.

Roots used were from young potted trees of yellow poplar (*Liriodendron tulipifera* L.), sweet gum (*Liquidambar styraciflua* L.), and large forest trees of shortleaf pine (*Pinus echinata* Mill.). Roots or root-segments were 3-6 inches long,  $\frac{3}{8}$ - $\frac{3}{4}$  inches in diameter, and 2-4 years of age. Since preliminary experiments had shown that little or no absorption by suberized roots occurs when young branch roots are present, any young branch roots that were present were not allowed to dip into the solution; in many instances the root-segments used were free from branch roots. Distal cut ends of roots were sealed with grafting wax to minimize the entrance of air and were not allowed to dip into the solution. Thus any colored solution absorbed by the roots must have entered through old roots. The experiment was repeated at least twenty times with yellow poplar and shortleaf pine; at least ten times with sweet gum.

After treatment, roots were dissected under low power binoculars and studied in cross section under a compound microscope. The red color of the dye could be seen easily in the root tissues of yellow poplar and sweet gum. In shortleaf pine, some roots contained so much natural red color in their bark that the color of the azosulfamide could not be detected. In such roots it became necessary to use other dyes. Light green and aniline blue proved reasonably satisfactory.

#### Results

Microscopic study of yellow poplar roots after treatment showed the presence of appreciable quantities of dye in the bark, while the wood re-

mained uncolored. The bark of yellow poplar is thick with a thin, fairly smooth periderm containing many lenticels. The accumulation of dye was observed in patches, not in uniform concentration throughout the bark. These patches could be traced to three sources: (a) lenticels, (b) breaks around the bases of branch roots, and (c) wounds.

The bark of pine roots is proportionately thinner than that of yellow poplar, with many overlapping plates of brittle periderm several inches long and about  $\frac{1}{8}$  inch wide. Some roots are invested with a fungus mantle; in these the plates of periderm seem much less brittle. When they were immersed in a dye solution under vacuum, suberized pine roots absorbed very little dye through the bark. Dissection showed that it tended to enter under the edges of the plates of periderm, but that the overlapping was sufficient to prevent the entrance of much dye solution into the living portion of the bark. When the plates of periderm were removed before treatment, large amounts of dye entered. Lenticels were not observed.

In every pine root studied, the presence of dye in the living portion of the bark could be traced to a small wound previously unnoticed. Little or no entrance was apparent around branch roots. This difference from yellow poplar seems to be the result of more complete coverage of the break in the old root around a branch root by overlapping layers of cork. Around the stumps of dead branch roots or the tips of abortive roots, a complete seal is effected by the formation of periderm. A similar seal has been described by PRESTON (5) for lodgepole pine.

A few observations of sweet gum suggest that in amount of absorption through suberized roots it lies between yellow poplar on the one hand and shortleaf pine on the other. Entrance seems to occur through wounds and in some degree around branch roots but not appreciably through lenticels. A more complete study of sweet gum roots is contemplated.

### Discussion

The old concept of absorption through only the young portions of root systems has seemed to several investigators (2, 3, 4) inadequate to account for the persistence of plants that transpire for long periods during which little or no elongation of roots occurs and when few, if any, young roots are present. Absorption through older suberized roots has been demonstrated experimentally: for sour orange by HAYWARD, BLAIR, and SKALING (2); and for dogwood, yellow poplar, and shortleaf pine by KRAMER (3). It seems likely that such absorption is important in the field, not only in evergreens but in a wide variety of deciduous trees and shrubs. The results of KRAMER (3) indicate that there are great differences in species as to rate of absorption through suberized roots.

An important factor in the potential rate of absorption by a given species is the anatomy of its roots. Since suberin is impermeable to water, a relatively thick suberized layer would render the root impervious to water except where breaks occurred in the suberized layer. Natural breaks occur (a) in

lenticels, (b) around the edges of overlapping plates of periderm, and, in most species, (c) around branch roots. In addition, wounds of various kinds may provide breaks in the suberized layer. The present experiments indicate that yellow poplar, through its lenticels and around its branch roots, absorbs water much faster than shortleaf pine which has few, if any, lenticels and which has an effective seal around its branch roots. These observations confirm the quantitative measurements of absorption by KRAMER (3).

### Summary

1. Entrance of water into suberized roots of yellow poplar, shortleaf pine, and sweet gum was studied by means of colored solutions. Roots were immersed in the solutions under vacuum, and the appearance of the dye in root tissues was taken as evidence of the entrance of water.

2. Microscopic examination showed that considerable quantities of dye, and therefore presumably of water, could enter old suberized roots and that there was an appreciable difference between different kinds of trees in the amounts that enter.

3. Much more dye was observed in yellow poplar than in shortleaf pine. Preliminary observations on sweet gum indicated that in amount of dye that enters suberized roots, it is intermediate between yellow poplar and shortleaf pine.

4. Three avenues of entrance were observed: (a) lenticels, (b) breaks around branch roots, and (c) wounds.

5. Comparative studies of roots of yellow poplar and shortleaf pine suggest that differences between kinds of trees as to capacity for absorption through suberized roots are attributable in part to anatomical characteristics of the bark of the roots, especially the number of lenticels and the completeness of the coverage by periderm of the inner, living portion of the bark.

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## NOTES

**Basil Elijah Gilbert.**—DR. GILBERT died June 27, 1945. He was born at Shedden, Ontario, Canada, on June 25, 1892. He was graduated from McMASTER COLLEGE in 1916 and subsequently served as an officer in the CANADIAN EXPEDITIONARY FORCES during WORLD WAR I. After the war he completed graduate work for the Master's degree at McMASTER COLLEGE in 1920. DR. GILBERT served as instructor of biology at BRANDON COLLEGE from 1921 to 1924. He then entered the UNIVERSITY OF CHICAGO and was awarded the doctorate in plant physiology in 1925. After graduation he joined the staff of the RHODE ISLAND AGRICULTURAL EXPERIMENT STATION at KINGSTON as a chemist. Since 1928 he served as director of research and was vice director of the station at the time of his death. DR. GILBERT published numerous important papers on phytochemistry, the nutrition of field crops and the inter-relationships of temperature and photoperiod. He was one of the original members of the AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS and also belonged to the AMERICAN ASSOCIATION for the ADVANCEMENT of SCIENCE, the AMERICAN CHEMICAL SOCIETY, and the BOTANICAL SOCIETY of AMERICA.

**Frontiers in Chemistry.**—Volume IV. Major Instruments and their Applications to Chemistry. Edited by R. E. BURK and OLIVER GRUMMITT. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 151 pages. \$3.50.

This is the fourth volume of the Frontiers in Chemistry lectures published under the auspices of Western Reserve University. With authoritative discussions by prominent scientists in the industrial field, the volume contains important contributions to chemical literature in the following subjects; electron diffraction and the examination of the surfaces; the electron microscope and its application; x-ray diffraction and its applications; chemical spectroscopy; the application of absorption spectra to chemical problems; and the infrared spectrometer and its application. Each article is expertly handled with figures and charts to elucidate the material. Complete bibliographies are also included with each chapter.

**Industrial Oil and Fat Products.**—ALTON E. BAILEY. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 735 pages, 111 figures. \$10.00.

The material in this monograph is well organized and concisely yet completely presented. With preliminary chapters on the structure and composition, the reactions, and physical properties of fats and fatty acids, subsequent chapters discuss specific fats and oils as to their characteristics and industrial utilizations. These include materials used in cooking and

salad oils, the plastic-shortening agents, butter and margarine, paints and varnishes, soaps, and other surface-active materials.

The final unit of the book comprises consideration of processes in fat technology. These include the extraction of fats and oils; refining and bleaching; deodorization; hydrogenation; soapmaking; fractionation of fats and fatty acids; fat-splitting, esterification, and interesterification; polymerization; isomerization; solidification; homogenation; and emulsification. Although the book is primarily for chemists, much important information concerning plant oils and their extraction will be of interest to physiologists.

**Physical Methods of Organic Chemistry.**—Volume I. Edited by ARNOLD WEISSBERGER. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 736 pages. \$8.50.

This volume has been prepared to aid the organic chemist in his research by eliminating the often burdensome task of searching periodicals for many physical methods necessary in his work. The book provides a description of tested methods, the theoretical background for understanding them, and the information necessary for a critical evaluation of the experimental results.

The chapters comprise contributions from twenty-seven scientists connected with leading research laboratories and universities. The subjects covered are melting and freezing temperatures, boiling and condensation temperatures, density, solubility, viscometry, surface and interfacial tension, monolayers and duplex films, osmotic pressure, diffusivity, calorimetry, microscopy, crystallo-chemical analysis, X-ray diffraction, electron diffraction, and refractometry. Numerous figures and bibliographical references are included with each topic discussed. A second volume by the same publishers will contain further chapters on this same topic with a subject index to both volumes.

**Life Membership.**—The Executive Secretary-Treasurer wishes to report the purchase of a Life Membership by CAPTAIN JOHN P. DECKER now of the Army Air Forces, Eglin Field, Florida. CAPTAIN DECKER received the Ph. D. degree from Duke University in June, 1942 and entered the Service a month later. He expects to be released soon and plans to return to Duke and continue work on the physiological aspects of pine—hardwood competition in the Piedmont forests. Since receiving his degree, two papers have appeared in PLANT PHYSIOLOGY under his name.

The purchase of a Life Membership or a Patronship is a definite investment in professional achievement and contributes to the welfare of the Society by ultimately increasing the General Endowment. We are happy to welcome CAPTAIN DECKER to our roster of Life Members and trust that his exemplary action may have a positive effect on others.







EDWIN CYRUS MILLER



# PLANT PHYSIOLOGY

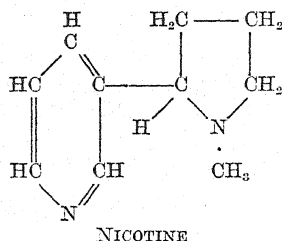
APRIL, 1946

## DEVELOPMENT OF SOME RECENT CONCEPTS IN THE PHYSIOLOGICAL CHEMISTRY OF THE TOBACCO ALKALOIDS<sup>1</sup>

RAY F. DAWSON

The plant alkaloids constitute a group of nitrogenous substances which possess great interest for the organic chemist because of their intricate molecular structure and for the pharmacologist because of the extraordinary physiological effects that many of them exert when introduced into the animal body. It is a regrettable fact, however, that so little is known of their physiological functions and of the mechanisms by which they are synthesized within the plant cell. Concerning these problems, the lack of an obvious experimental approach has led to extensive speculation. For instance, PICTET, who first synthesized nicotine, assumed that the alkaloids may represent degradation products of proteins, nucleic acids, and chlorophyll (35). The great JUSTUS VON LIEBIG implied their use as substitutes for the mineral bases within the plant body when he developed his "mineral theory" (3, 26). The most prevalent view at the present time has been well expressed by CROMWELL (5) and by VICKERY (42). This hypothesis holds that the alkaloids represent by-products of nitrogen metabolism in which irreversible reactions are involved and that they differ from the end-products of nitrogen metabolism in animals by their retention within the body of the organism.

Within the past decade a serious attempt has been made to ascertain the nature of nicotine production in the tobacco plant. Several factors have contributed to this choice, but the most important have undoubtedly been the



worldwide use of nicotine as a mild narcotic and the relative ease with which the alkaloid may be quantitatively assayed. From these investigations cer-

<sup>1</sup> The ninth STEPHEN HALES Address. 1945.

tain well-defined concepts have been developed concerning 1) the locus of nicotine synthesis, 2) the extent of translocation, and 3) the nature of nicotine accumulation in the growing plant body (9, 14, 19, 32). It has seemed worthwhile to retrace here the development of these concepts in order to call attention to the important new opportunities which they provide for basic research in the physiological chemistry of nicotine in particular and of the plant alkaloids in general.

### Distribution of nicotine in the growing plant

The very excellent work of VICKERY and his collaborators (43, 45) has provided a picture of the accumulation of nicotine in the leaf and stem of the tobacco plant during its growth and developmental stages. The general pattern of accumulation begins with the seedling, nine to eleven days after germination, for the mature tobacco seed does not contain detectable quantities of the alkaloid. When the young plants have attained transplanting size, the differential nature of alkaloid distribution within the plant body has already become apparent. That is, nicotine is present in appreciable quantities in both root and stem, but by far the greatest proportion resides in the leaves. Furthermore, the proportion of the total nicotine of the plant to be found in the leaves throughout most of the period of growth (45) remains within the extremely limited range of 85 to 88 per cent. As VICKERY has pointed out, "The constancy of the proportion in the leaves is remarkable in view of the rapidly changing relative proportions of leaf and stem tissue as the plant grows, and raises an interesting question with regard to the kind of cells that are capable of synthesizing nicotine." It is also of interest to note that the most rapid rate of overall nicotine accumulation may occur at a time in the life of the plant when growth in terms of dry weight increase has all but ceased (45). Clearly, therefore, nicotine accumulation, in the aerial organs at least, is not directly associated with the growth processes of the tobacco plant. This conclusion has, indeed, already been expressed by MOTHES (31). As VICKERY has suggested, the interpretation of these and of other aspects of nicotine accumulation in tobacco, particularly the quantitative relationship between nicotine nitrogen and total nitrogen in the leaf, is difficult "in view of our ignorance of the exact position of nicotine in the scheme of nitrogen metabolism in the tobacco plant."

Certainly, one of the important contributions of VICKERY and his associates lies in their emphasis upon the lack of information concerning the "kind of cells," that is, the exact locus or loci within the plant body where nicotine formation can occur. Hence it becomes apparent that the dilemma of interpretation can be dispelled only by going back to some more fundamental problems among which is the ultimate origin of nicotine.

### The locus of nicotine formation

The first extensive physiological investigation of nicotine formation in tobacco was carried out by MOTHES (31). Working within the framework

of older suppositions concerning the *in situ* nature of alkaloid synthesis and the doctrine of phloem-transport of organic compounds, MOTHES attempted to alter or to block nicotine formation by manipulations of the environment of the plant. He found the process of nicotine accumulation to be an extremely stubborn object for experimentation. So long as the leaf was attached to the growing plant, it continued to acquire nicotine, even under such drastic conditions as nitrogen deprivation and carbohydrate starvation. No appreciable transport of alkaloid from the leaf could be detected. It seems reasonable to conclude, therefore, that the nicotine metabolism of the tobacco leaf is characterized by a ponderous stability and that the usual methods of physiological experimentation can not be expected to yield information of much value.

The methodology of experimental morphology would seem to offer more promise. It has been noted above that as long as the tobacco leaf is attached to the growing plant, nicotine accumulates regardless of environmental circumstances. MOTHES observed, however, that excised leaves ceased immediately to acquire the alkaloid in spite of the nature of their subsequent treatment. This phenomenon of accumulation interrupted by excision has, of course, long been known to occur in tobacco leaves which are stripped from the plant preparatory to processing for commercial use. The work of MOTHES and the researches of VICKERY and PUCHER (44, 46) on the chemical changes that occur in excised leaves during culture under a wide variety of conditions have, nevertheless, brought into bold relief the simple fact that organic connection between leaf and plant is necessary to continued increase in the nicotine content of the leaf.

In logical continuation of this observation, the following points have been experimentally established. First, when tobacco shoots are excised and cultured in water, they, likewise, cease at once to accumulate nicotine (7). Secondly, if segments of tobacco stems are excised and placed in moist chambers, they send out lateral branches which are nicotine free (10). Thirdly, excised segments of tobacco stems placed in moist chambers also produce callous tissue at their basal surfaces. This callous tissue contains no alkaloid (10). Fourthly, the callous tissue that develops on the cut ends of the petioles of excised leaves held in moist chambers contains nicotine, but this nicotine is transported from the leaf blade and hence does not lead to an increase in the total amount of alkaloid in the leaf (unpublished data). Finally, nicotine does accumulate in extraordinarily large amounts in excised leaves that have been rooted in sand (9). As a result of these experiments, it is possible to conclude that not only is an organic connection between leaf and plant necessary for continued alkaloid accumulation, but also the plants (or the leaf) must bear roots. Consequently, it becomes necessary to look to the root for the source of nicotine or of its precursor(s).

NATH in India (33) and BERNARDINI in Italy (1) were among the first to report the remarkable results to be obtained by grafting tobacco upon tomato. These authors observed a great decrease in the concentration of



nicotine in the leaves of the tobacco scions. NATH reported the equally interesting observation that the reciprocal graft (i.e., tomato scion on tobacco stock) accumulated nicotine in both stock and scion. A year later HASEGAWA (18) confirmed these results. Subsequently, EVTUSHENKO (15), KUSMENKO and TIKHVINSKAYA (25), SHMUCK (37), SHMUCK, SMIRNOV and ILYIN (39), and SHMUCK, KOSTOFF, and BOROZDINA (38) brought forth extensive confirmation based upon grafts of tobacco with tomato and with a variety of other species. The experimental data collected by these investigators, fortunately, were characterized by more agreement than were the interpretations applied to them. The earlier report by NATH and the observations of BERNARDINI and HASEGAWA were based upon data too meager to permit more than speculation concerning their significance. It is difficult to understand, however, why the far more extensive and well planned work of SHMUCK and his colleagues was regarded by them to support the mystical notion of KRENKE (24) concerning the release of "hidden properties" within the scions under the influence of "new developmental conditions." SHMUCK definitely discounted the possibility of translocation of the alkaloid from root to shoot (38, 39).

More recent investigations (10) have considerably simplified the problem of interpretation. Tobacco stocks and scions were defoliated and cut as short as possible in order to reduce materially their initial content of nicotine. They were then reciprocally grafted with tomato. The tobacco leaves that subsequently developed upon tomato stocks were nicotine-free with the exception of the lowermost, which contained traces of the alkaloid. From the upper stem and the inflorescence of the tobacco scion there was isolated a base which could be determined as "nicotine" by the usual silicotungstic acid precipitation but which was not identical with this alkaloid. On the other hand, nicotine did accumulate extensively in tomato scions grown upon tobacco stocks. Such accumulation was greatest in the lower and older leaves, and extensive injury occurred to the tissues of these leaves. One of the most interesting observations, however, in connection with the problem of the origin of nicotine is found in the sectoral nature of nicotine accumulation in the tomato component of approach grafts of tobacco with tomato (10). Such a response could indicate only an upward transport of nicotine through the xylem. This view was confirmed by the isolation of nicotine from the sap that bleeds from the cut stumps of decapitated tobacco plants. The latter observation, likewise, demonstrated that it is nicotine itself that is transported to the leaf and not some precursor which can be converted to nicotine once it has reached the leaf cells. These findings have since been confirmed completely by the work of MOTHES and HIEKE (32) and in large part by the subsequent report of PAL and NATH (34).

To recapitulate, the evidence obtained from excised organs and from reciprocal grafts with non-alkaloid producing species has shown that nicotine is not produced *in situ* in the leaf and stem of the tobacco plant but rather is translocated to these organs there to accumulate.

The next question, of course, concerns the actual locus of the nicotine synthetic mechanism within the plant body. The absence of nicotine in tobacco stems and leaves when grafted close to the roots of tomato stocks obviously suggests that the alkaloid is formed only in the roots of the tobacco plant. Final proof for the correctness of this view was obtained when nicotine was isolated from both the tissues and the spent culture fluids of excised tobacco roots in sterile culture (11).

Many heretofore unexplained characteristics of nicotine content in tobacco now seem susceptible of interpretation. Among these may be mentioned the differential nature of alkaloid accumulation in leaves and stems during growth (45); the extensive enrichment of the leaves of topped tobacco plants with respect to nicotine; the low nicotine content of the rapidly grown sucker or ratoon crop (30); the abrupt termination of alkaloid accumulation in excised tobacco leaves and shoots; and the difficulty encountered by MOTHES in his experiments with respect to altering the nicotine content of the leaves of intact plants by manipulating those environmental factors which influence principally the aerial shoot.

#### The extent and nature of nicotine transport

Evidence was presented in the preceding section to show that nicotine is translocated from root to shoot and that the pathway for at least the bulk of such transport is undoubtedly the xylem. It would seem reasonable to assume, therefore, that an examination of the distribution of the alkaloid in various parts of the stem and in leaves at different levels on the stem might reveal the major currents of such transport. MOTHES (31) reported, and indeed general experience shows, that in the absence of senescent changes the total nicotine content per leaf decreases with increase in height of the leaf position on the stalk. That is, so long as they remain anabolically active, the older leaves contain the greatest quantities of alkaloid. The distribution of nicotine in the stems has also been examined (9). In this case, the greatest accumulation of the alkaloid is found in the cortex, although the pith and xylem contain appreciable amounts. Taking into consideration the additional fact that by far the greater proportion of the total nicotine of the aerial shoot is located in the leaves, it at once becomes apparent that the patterns of nicotine distribution within the shoot are identical, at least qualitatively, with what might be expected if the alkaloid were translocated in the transpiration stream. To state the matter in another way, the alkaloid can be considered to accumulate in the different regions of the shoot in proportion to the anticipated temporal duration and relative intensity of their transpirational losses. Based upon this viewpoint, a flow-diagram of nicotine transport and deposition within the plant body may be constructed with interesting results. It may be noted that such a diagram predicts the differential character of nicotine distribution in the tobacco leaf as determined experimentally by CICERONE and MAROCCHI (4).

At this point it seems desirable to raise the question of the state in which

nicotine occurs in the cells and cell fluids. In the above discussion, it has been assumed that the sole forces governing nicotine distribution within the plant body are diffusion and transpiration. If, however, nicotine were to associate electively in salt formation with any particular acidic substance or substances and this were to be followed by the appearance of a solid phase, then it might be expected that the foregoing interpretation of the nature of nicotine accumulation would be considerably in error. Almost without exception, texts and reference books contain the statement that nicotine is found in tobacco leaves as the salt of malic and citric acids. Now, the alkaloid is a relatively feeble di-acid base (20) and would be expected to undergo salt formation readily. In the very low concentration in which it occurs in the leaf tissue fluids (0.012 molar or approximately 0.20 per cent. in the experiments of VICKERY and PUCHER (45) at the 75-day collection), it is not likely that precipitation of nicotine as the salt of an organic acid would take place. On the contrary, since most of the salts of nicotine are readily soluble in water, it is far more likely that the alkaloid exists in the living cell in a complex series of equilibria with negatively charged particles any and all of which may undergo extensive changes in relative concentration with drift in time and in metabolic activity. Therefore, nicotine cannot be said to associate electively with any one or two acids but must be thought of as entering into the general buffering system of the plant cell. Indeed, to the limited extent that its low concentration in terms of normality in the cell sap permits, it may be said that its chemical properties demand recognition of such a function for this alkaloid in tobacco physiology.

#### The nature of the nicotine synthetic process

As an approach to the highly interesting but largely undocumented subject of the intermediary metabolism of nicotine, it is necessary to observe that the localization of the synthetic mechanism with respect to specific tissues or developmental zones within the tobacco root has not yet been investigated. Likewise, information concerning the nature of the environmental factors which can influence the rate of synthesis is meager and often conflicting. It seems fairly certain, however, that the extent of accumulation in the leaf and presumably, therefore, the overall rate of production by the root can be increased by growing the plants in a heavy soil as contrasted with a light soil (private communication), in a dry soil as compared with a moist soil (6), and by topping and suckering the plants during the growth period (30). Interpretation of these results is greatly complicated by the lack of suitable data on concomitant shifts in root-shoot ratio. The same may be said of the results obtained from the application of nitrogenous fertilizers (31).

Evidence has been obtained (45) which indicates that nicotine disappears as such during the profound redistribution of metabolites that occurs within the plant body following the onset of sexual reproduction. In the last stages of growth, therefore, the rate of nicotine accumulation in the

plant as a whole is determined by the progressive difference between the rate of synthesis and the rate of utilization or breakdown. This differential is probably augmented by a simultaneous diversion of the carbohydrate output of the leaves from root to inflorescence. Indeed, many observations (unpublished) point to the necessity of continued and adequate supplies of carbohydrates in maintaining maximum rates of nicotine output by the roots. In this connection, the remarkable changes that take place as a result of topping tobacco plants during the growth period are of interest. In such cases, there are no reproductive structures or fruits to monopolize the food supply. The result is that the leaves become greatly enlarged due to abnormal increases in the size of the parenchymatous cells (49). Associated with this change is usually a very considerable increase in alkaloid concentration (30). The magnitude of such increase seems to be greater than would be expected on the basis of an increase in the root-shoot ratio alone.

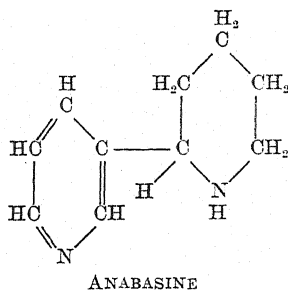
Aside from circumstantial evidence concerning the necessity of ample supplies of carbohydrate, nothing is known concerning the influence of temperature, oxygen tension, nitrogen supply, and similar variables on the absolute rate of nicotine synthesis in the roots. This field obviously provides much opportunity for future research.

In connection with the identification of possible intermediates in alkaloid synthesis, TRIER (48) has suggested a purely hypothetical and rather improbable scheme which would yield the basic ring structure of nicotine by the simultaneous oxidative decarboxylation of proline and nicotinic acid. KLEIN and LINER (23) have published data to show that excised tobacco leaves can make nicotine from proline alone. GORTER (17) repeated these experiments and, using a somewhat different method for the expression of data, failed to confirm the results. A third investigation of the problem has also been reported (7) in which even larger increases were obtained by feeding nicotinic acid to excised tobacco shoots than were obtained by feeding proline. The recent discovery that nicotine is not normally produced in important amounts in tobacco leaves and the failure of the investigators cited above to establish the validity of analytically determined increases in nicotine content by suitable statistical controls necessitates the adoption of a somewhat skeptical attitude toward such results. Although it seems very unlikely, the possibility cannot be denied that leaves may be found to manufacture at least small amounts of nicotine provided suitable intermediates in the total synthesis are supplied by the investigator and/or by the root of the intact plant. In this connection, a repetition of the experiments described above utilizing naturally nicotine-free tobacco leaves from appropriately grafted plants should clarify once and for all the position of nicotinic acid and proline in the alkaloid metabolism of the aerial organs.

VICKERY (41) has attempted to identify the possible precursors of nicotine by another approach. Rather than feed the plant with hypothetical intermediates, this investigator resorted to direct isolation from the seed meal of substances that might play a part in the formation of nicotine during



tine synthesis in *N. tabacum* is usually far more intensive than is the production of anabasine in *N. glauca* (40). If it had been true, this relationship would have intimated, among other possibilities, a competition between the synthetic mechanisms of nicotine and anabasine for a common precursor. The stimulating prospect of discovering such a system has been eliminated by the demonstration (14) that anabasine formation does not predominate in the genetical sense over nicotine production in the various combinations



described above. Rather, the extensive secondary conversion in these combinations of nicotine to nornicotine and the difficulty encountered in identifying the latter substance in the presence of anabasine have contributed to erroneous interpretations. It should be noted that the demonstration of independent anabasine synthesis in both root and shoot of *Nicotiana glauca* (12) has shown that the total synthesis of alkaloids is not an inherently unique property of root tissues.

#### The physiological significance of nicotine

Insofar as it is justifiable to attempt to blueprint the physiology of the tobacco plant and to assign a function to each of the constituents thereof, it would seem well to list the more obvious alternatives with respect to nicotine. For instance it may be possible that nicotine originates as a by-product of a number of irreversible and physiologically useless reactions. Or it may be that it is formed for any one of several reasons as a by-product of chemical reactions that do play a rôle in cell metabolism. Again, the final steps in the synthesis of the alkaloid molecule may involve reactions of use to the cell; or the finished product may itself participate in important activities which are not at present recognized.

It will be noted that the views expressed prior to 1942 were based upon the assumption that nicotine is produced largely in the green leaf and that its physiological import must, therefore, be linked in some way with this organ. From this point of view, the great variation that occurs in nicotine content of different strains and crops of tobacco; the fact that tobacco scions can grow and develop quite normally on tomato stocks without more than minute traces of available nicotine; and the failure of nicotine fed through the cut stems of excised tobacco shoots to alter nitrogen metabolism appreciably (8), all support the conclusion that perhaps nicotine plays no important



rôle in the aerial organs. It is obvious, therefore, that an investigation of the effects of nicotine upon the roots of the tobacco plant is necessary. The results of some preliminary experiments, as yet unpublished, are available and are outlined here solely for the purpose of indicating the direction which future investigations of alkaloid physiology are expected to take in this laboratory.

In these experiments, Connecticut Broadleaf tobacco plants were grown in sand culture with a mineral nutrient solution that contained nitrogen only in the form of nitrate for approximately seven weeks. At that time, one-half the plants were given a supplement of 0.10 per cent. nicotine as the hydrochloride. This treatment was maintained for ten days: one-half of the nicotine-fed plants and one-half of the control plants were then harvested. For another nineteen days the remaining plants were watered only with tap water and received no more nutrient solution. These were likewise harvested. The results were rather astonishing.

In the first place, the roots of the plants to which nicotine was supplied very quickly acquired a violet-blue pigmentation which appeared to be localized in the vacuoles of the external cortical cells. No root injury of any sort was observed although the coloration was rather intense and in great contrast to the light cream color of the roots of the control plants. Some of the pigment was obtained in aqueous solution. It was insoluble in fat solvents, unchanged by zinc dust in dilute acid, but was destroyed by strong acid and strong base. The addition of silicotungstic acid to a portion of the original solution immediately led to destruction of the color and to the separation of a voluminous white precipitate. The supernatant solution was clear and colorless.

The second remarkable feature of these plants was the accumulation during the initial ten-day period of large amounts of nitrate nitrogen in the leaves and stems. Accompanying, or perhaps resulting from the accelerated nitrate uptake, was a proportionate increase in the reduced forms of nitrogen in root, stem and leaf (table I).

The plants that were supplied with tap water for a subsequent period of nineteen days gave further evidence of the changes that had been brought about. Under these conditions, the excess accumulation of nitrate largely disappeared, and a corresponding increase in other forms of water soluble and in hot-water-insoluble nitrogen fractions resulted. It was noted that the overall rate of nitrate assimilation during the period of nitrogen deprivation greatly exceeded that for ammonia assimilation (cf. 47).

It was observed that the roots of the nicotine-fed plants accumulated relatively much ammonia and amide nitrogen, whereas the amount of nitrate stored in these organs did not appreciably change. Clearly, the overall response to the presence of extra nicotine, so far as nitrogen metabolism is concerned, was the absorption by the roots of abnormally large amounts of nitrate nitrogen from the nutrient solution.

If the view is adopted that nicotine may function within the tobacco root as an accelerator of nitrate absorption, it then becomes necessary to inquire

into the possible mechanisms through which such an activity might be exercised. From the physiological point of view, alkaloid synthesis could conceivably increase the concentration of hydroxyl ion derived from water and thereby make possible increased acid exchange between root and soil. Furthermore, the alkaloid could serve as a buffer substance in the roots against the accumulation of dangerous amounts of nitric acid or of its immediate reduction product, nitrous acid. These suggestions break down under the fact that the ratio of nicotine to nitrate absorbed was so exceedingly small. For instance, in the experiment under consideration, only 1.2 m.e. of nicotine (calculated as mono-acid base) were absorbed from the substrate and accumulated without change, while 28.7 m.e. of nitrate were

TABLE I

CHANGES IN THE NITROGENOUS FRACTIONS OF TOBACCO PLANTS AS A RESULT OF (1) THE ADDITION OF NICOTINE HYDROCHLORIDE TO THE NUTRIENT SOLUTION FOR TEN DAYS AND (2) SUBSEQUENT WITHDRAWAL OF NICOTINE AND OF MINERAL NUTRIENTS FOR NINETEEN DAYS. FIGURES ARE IN MILLIGRAMS OF NITROGEN PER PLANT

	NITROGENOUS FRACTIONS					
	NH <sub>3</sub> -N	AMIDE-N	NO <sub>3</sub> -N	NICO-TINE-N†	UN- KNOWN-N	INSOLU- BLE-N
	mg.	mg.	mg.	mg.	mg.	mg.
(1) Nicotine supplied*						
Shoots .....	+ 20.2	+ 59.0	+ 119.4	+ 32.4	+ 35.8	+ 80.4
Roots .....	+ 13.1	+ 9.2	- 0.4	+ 1.0	+ 63.5	
(2) Nicotine withdrawn†						
Shoots .....	- 7.4	+ 4.0	- 246.2	+ 2.8	+ 128.6	+ 83.0
Roots .....	- 11.3	- 6.1	- 1.0	+ 0.6	- 38.9	

\* Figures express the differences between plants receiving nicotine for ten days and those receiving none.

† Figures express the differences between plants from which nicotine and all mineral nutrients were withdrawn for nineteen days and plants which had been supplied with both nicotine and mineral nutrients for an earlier ten-day period.

‡ During the period of nicotine feeding, each plant received 86.5 mgm. of nicotine nitrogen in 500 ml. of nutrient solution per day.

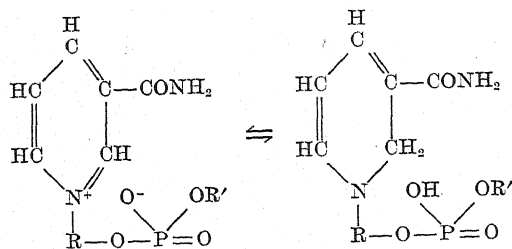
obtained from the same source (the solution contained no ammonium nitrogen).

A second possibility would seem to be that nicotine may alter the permeability of the root tip cells to the calcium, magnesium or potassium salt of nitric acid. This effect should be readily detected by ash analyses of the root, stem, and leaf tissues of the plants concerned.

The most suggestive possibility, however, would seem to follow the observation (table I) that, while much nitrate presumably traversed the root on its way to storage in leaf and stem, none was accumulated by the root cells as such. Instead, these cells contained relatively large amounts of ammonia, amide, and the undetermined forms of nitrogen including protein. While no estimate of the actual intensity of nitrate reduction in the roots is available from the existing data, it seems not unlikely that the presence of extra nicotine may have resulted in a greatly increased rate of reduction of nitrate to ammonia in the roots. This, in turn, may have conditioned an increase in

the rate of nitrate absorption by providing not only an extra supply of hydroxyl ion for acid exchange with the nutrient solution but also an equivalent amount of ammonium ion for preserving a favorable pH within the root cell fluids.

It seems worthwhile here to point out some of the chemical properties of nicotine, by means of which effects such as those described above could be brought about. For instance, the nicotine molecule contains two feebly basic trivalent nitrogen atoms through which it is entirely conceivable that salt linkages might serve as points of attachment to specific proteins. The pronounced narcotic action of nicotine in the animal body lends weight to such a suggestion and, indeed, indicates the need for an investigation of the behavior of the alkaloid in both the animal and the plant cell from the point of view of the systems described by JOHNSON, EYRING, and WILLIAMS (22); JOHNSON, EYRING, and KEARNS (21); and MCELROY (29). If the narcotic action of nicotine in the animal body is based upon its ability to displace reversibly the prosthetic groups of one or more enzymes or upon its ability to denature reversibly the protein component of such enzymes, then the prospect must also be envisaged that nicotine or a metabolite of nicotine in

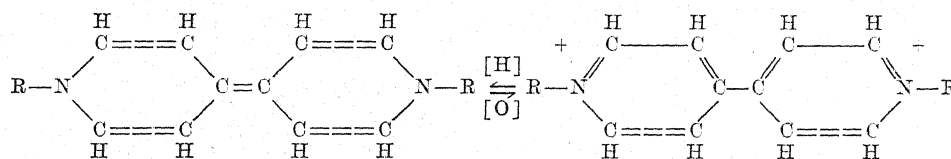


SCHEME II

combination with a specific protein may either catalyze or inhibit physiologically useful reactions in the tobacco root cells. In the event that evidence were forthcoming for such a mechanism, however, it would also be necessary to explain the fact that so much of the nicotine is lost from this combination and is permitted to be carried in the transpiration stream to those parts of the plant in which its physiological activity seems to be at a minimum [cf. the dissociation of nicotinamide-protein systems (36)].

The possible combination of nicotine (or of nicotine metabolites) with specific proteins raises many interesting possibilities for experimentation. For instance, although WARBURG has studied the properties of heme in combination with nicotine, no one seems to have attempted to obtain a biologically active substance by substituting nicotine for nicotinic acid amide in model substances related to the codehydrogenases. The codehydrogenases themselves are derivatives of pyridine, and their enormous biological importance needs no emphasis here (36). It is of interest to note, however, that the pyridine ring nitrogen of these compounds seems peculiarly fitted for the facile change from trivalency to pentavalency and back again as electrons are shifted from one catalyst to another in the course of cellular respiration (Scheme II). The general properties of pyridinium compounds and the

possibility of the existence of semiquinoid intermediates (27) in the reversible hydrogenation of nicotinic acid amide lead to the suggestion that, even in the absence of established combination with specific proteins, the potential biological importance of pyridine derivatives including nicotine deserves more widespread attention. From the point of view of the present discussion, the formation of quinoid structures such as those of the blue-colored N,N-dialkyldihydrodipyridyls is of great interest (16). The latter are easily oxidized with silver nitrate and less readily with atmospheric oxygen, followed by treatment with hydrochloric acid to form the dialkyl halogenide of 4,4'-dipyridyl. These, in turn, may be reduced back to the original blue mother compound with zinc dust and glacial acetic acid (Scheme III).



SCHEME III

While it seems likely that the pyridine ring in the nicotine molecule could contribute to such biochemical processes as the reduction of nitrate, it must not be overlooked that the pyrrolidine ring also possesses interesting properties. For instance, nicotine can be oxidized by mere exposure to air and to sunlight to the corresponding pyrrolidine-N-oxide containing a pentavalent nitrogen atom. With silver oxide in warm water, nicotine is dehydrogenated to nicotyrine (2). To carry speculations such as these to their logical conclusion, it becomes necessary to assume that electron shifts, in which nicotine or a metabolite of nicotine participates, could result either directly or indirectly in the absorption of relatively large amounts of nitrate ion from the soil solution without disturbing the electrostatic balance of the root tissue fluids. Whether or not this alkaloid, or any other alkaloid, can perform such a direct function in the plant that produces it remains to be determined by actual experimentation. These relationships are suggested here merely to call attention to the fact that the field is by no means devoid of problems for investigation.

Finally, it seems possible that careful study will reveal equally interesting but relatively minor biological rôles for nicotine in tobacco stem and leaf tissues. For instance, it is now definitely established that a certain small fraction of the nicotine content of excised leaves disappears as such during curing and during culture (44, 46). The extensive mobilization and disappearance of nicotine during fruiting and senescence has been noted above (45). Experiments have also been performed in which nicotine was supplied to excised tobacco shoots during culture in light (8). In all cases, approximately 25 per cent. of the nicotine absorbed by these shoots was converted into some other form of nitrogen which was water-soluble but not volatile with steam from alkaline solution. Certainly, the chemical nature

of the alkaloid would seem to fit it for many possible rôles in cellular metabolism including oxidations and reductions, both reversible and irreversible, and combinations with other compounds such as the proteins. It is, therefore, a great mistake to relegate it without further study to the scrap-heap of "waste-products" of tobacco metabolism and thence to consider the matter of little further importance.

The author is greatly indebted to DR. W. G. FRANKENBURG, Director of Research for the General Cigar Company, for his kindness in critically reviewing the manuscript prior to its publication.

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## NEW CARRIERS FOR PLANT GROWTH REGULATORS<sup>1</sup>

R. B. WITHROW AND F. S. HOWLETT

Lanolin has been used extensively as a carrier for indolebutyric acid for the production of parthenocarpic tomato fruits and for supplementing pollination and fertilization in the tomato. However, lanolin pastes are tedious to apply, they leave a sticky residue, and, in the early spring, the red or pink color does not develop evenly where such residues are excessive. Fruits produced following application of lanolin pastes and particularly lanolin emulsions are especially susceptible to the physiological disorder, blossom-end rot.

The writers have been concerned with possible substitutes for lanolin and for the emulsifying agent employed in the standard lanolin paste and lanolin-triethanolamine stearate emulsion (table IV). The ideal carrier for any growth-regulating substance should have the following characteristics: (1), low physiological activity in relation to the plant tissues involved; (2), physical stability so that the rate of release of the regulator is not greatly influenced by variations in environmental conditions such as temperature and moisture; (3), capacity to hold sufficient concentration of the regulator for optimum results, without the formation of large crystals or the occurrence of other forms of physical separation; (4), chemical inertness to the growth regulator so that the regulator is not chemically changed by the presence of the carrier; (5), ease of application to limited portions of the plant; and (6), low cost.

The procedure followed has involved the application of indolebutyric acid in various carriers to flowers of Globe Strain A tomato, both in the production of seedless fruits as well as in supplementing pollination and fertilization. In this report, only those carriers will be discussed which have given results comparable to those given by the standard lanolin emulsion. The bases of comparison used included (1) proportion of flowers setting fruit and the size attained by the fruits, (2) proportion of fruits from treated flowers developing blossom-end rot, (3) extent of the development of gelatinous pulp in the carpels, and (4) the ripening of such pulp. Data on the application of these carriers and fruit set occurring with them will be published in a subsequent paper.

### Tissue injury tests with hydrocarbons, waxes, esters, and oils

In the search for substitutes for lanolin, tissue injury tests of a large number of hydrocarbons, waxes, esters, and oils were made on the leaves of tomato and soybean. Data on these tests, together with some of the properties of the various materials, are presented in table I.

<sup>1</sup> A joint contribution from the Purdue University and The Ohio Agricultural Experiment Stations. Journal Paper no. 204, Purdue University Agricultural Experiment Station.

Lanolin, at temperatures of 70° F. and below, caused no injury. At temperatures above 100° F., the leaves appeared oil-soaked and the tissues later died. Vaseline soaked into the leaf tissues a little more rapidly than lanolin and was somewhat more injurious at high temperatures. At low temperatures, it did not cause any injury. Paraffin oil killed the tissues and

TABLE I  
PROPERTIES OF WAXES AND SIMILAR MATERIALS AS CARRIERS FOR INDOLEBUTYRIC ACID (IB) IN POLYVINYL ALCOHOL

WAX OR OIL	SOLVENT PROPERTIES FOR INDOLEBUTYRIC ACID	POLYVINYL ALCOHOL EMULSION STABILITY	TISSUE INJURY	APPROXIMATE SOLIDIFYING POINT	REMARKS
Carnauba wax	Good	Very stable	None	°C. 85	Good carrier
Beeswax, white or yellow	Fair	Stable	None	62	Low activity of IB
Opal wax	Very good	Very stable	None	87	Low activity of IB
Wax blend no. 1	Very good	Stable	None	78	Good carrier
Wax blend no. 2	Very good	Stable	None	73	Good carrier
Spermaceti	Very good	Unstable	None	45	Good in mixtures with higher m.p. waxes
Lanolin, anhydrous	Good	Slightly unstable	Slight	30-40	Fair, except at high temperatures
Solid hydrocarbons	Poor	Stable	None	..... 65	Poor solvents for IB
Petrowax A					
Paraffin					
Ceresin					
Ozokerite					
Vaseline	Poor	Stable	Slight	.....	Poor solvent for IB
Paraffin oil	Poor	Stable	Severe	.....	Poor solvent for IB
Glyco waxes	Poor	Stable	.....		Poor solvents for IB
Glycerol esters	Good	Stable	Slight to severe	25	Injurious as a class -
Cocoa butter					
Bayberry wax				..... 43	
Beef tallow					
Glycerol monostearate S,					
Glyco					
Cocanut oil					
Olive oil					
Corn oil					
Cetyl alcohol	Very good	Unstable	None	49	Good in mixtures with other waxes
Stearic acid	Good	Unstable	Severe	69	

produced a water-soaked appearance. The solid hydrocarbons such as Petro-wax A, paraffin, ceresin, and ozokerite resulted in no injury when mixed with sufficient lanolin for easy application to the leaves.

The true waxes such as carnauba, beeswax, and spermaceti caused no injury. Opal wax, a hard glycerol ester, was also non-injurious. The

glycerol esters such as cocoa butter, bayberry wax, beef tallow, and glycerol monostearate, induced varying degrees of injury when applied in mixtures with lanolin. Coconut, olive, and corn oils were very injurious to leaf tissues. Cetyl alcohol was quite inert and harmless.

#### Solubility for indolebutyric acid and other data for hydrocarbons, waxes, esters and oils

The solubility of indolebutyric acid was determined by adding the acid at the rate of 1.0 to 10 gm. of the material heated to about 100° C. If the crystals did not dissolve readily, the solubility was considered as low. Vaseline and the liquid and solid hydrocarbons are poor solvents for indolebutyric acid; the true waxes and glycerol esters are good solvents for indolebutyric acid. Beeswax and opal wax appeared to react with the indolebutyric acid so that the effectiveness of the acid was reduced in setting fruit.

#### Tissue injury tests with emulsifying agents

The data on tissue injury for a number of emulsifying agents are given in table II. The various grades of polyvinyl alcohol, the alkaline and acid

TABLE II

CHARACTERISTICS OF VARIOUS MATERIALS AS EMULSIFYING AGENTS FOR WAXES CONTAINING INDOLEBUTYRIC ACID (IB)

EMULSIFYING AGENT	EMULSIFYING PROPERTIES	TISSUE INJURY	TYPE OF EMULSION
Triethanolamine stearate	Excellent	Toxic*	Alkaline
Polyvinyl alcohol, high viscosity grade DuPont, PVA, RH-403	Very good	Nontoxic	Acid or alkaline
Polyvinyl alcohol, medium viscosity grade DuPont, PVA, RH-488	Good	Nontoxic	Acid or alkaline
Polyvinyl alcohol, low viscosity grade DuPont, PVA, RH-623	Very poor	Nontoxic	Acid or alkaline
Gelatin, pharmaceutical alkaline grade, Pharmagel B	Very poor	Nontoxic	Alkaline
Gelatin, pharmaceutical acid grade, Pharmagel A	Poor	Nontoxic	Acid
Methyl cellulose, low viscosity grade Dow Methocel, 15 cps	Poor for waxes; good for oils	Nontoxic	Acid or alkaline for oils only
Methyl cellulose, high viscosity grades Dow Methocel, 400 and 4000 cps	Poor	Nontoxic	
Bentonite clay	Poor	Nontoxic	Alkaline; leaves objectionable residue

\* Injury tests determined with undiluted triethanolamine stearate. Toxicity not apparent when applied at low concentrations mixed with inert materials.

grades of pharmaceutical gelatin, high- and low-viscosity methyl cellulose, and Bentonite clay were all inert and produced no significant injury to leaf tissues when applied as aqueous mixtures. Triethanolamine stearate was very injurious to leaf tissue when applied undiluted. The components triethanolamine and stearic acid were also very injurious when applied separately.

#### Emulsification properties of agents

The low viscosity grades of methyl cellulose (4) form stable emulsions with oils but not with waxes. Methyl cellulose precipitates from solutions at the melting points of the waxes used. Bentonite clay does not form stable emulsions at concentrations sufficiently low to prevent objectionable residue on the fruit. Pharmagel A, derived from an acid-treated gelatin precursor, also formed unstable emulsions with the waxes used.

The high viscosity grades of polyvinyl alcohol and the pharmaceutical gelatin derived from an alkali-treated precursor, Pharmagel B, were the best

TABLE III

COMPOSITION OF CARNAUBA WAX AND LANOLIN MIXTURES

WAX NO.	COMPOSITION
1	Carnauba wax ..... 45% Lanolin, anhydrous ..... 50% Cetyl alcohol ..... 5%
2	Carnauba wax ..... 25% Lanolin, anhydrous ..... 70% Cetyl alcohol ..... 5%

emulsifying agents for the waxes. Polyvinyl alcohol as an emulsifying agent is described by E. I. duPont de Nemours and Company (5, 6). The production of emulsions with pharmaceutical gelatins is described by TICE (2, 3) and the formulae given here involve the proportions of alkali recommended by him for Pharmagel B emulsions.

#### Emulsions

The blended waxes given in table III were the most suitable for the formation of emulsions. Carnauba wax used alone resulted in emulsions of relatively high viscosity. The two mixtures, wax blend no. 1 and wax blend no. 2, formed low-viscosity sprayable emulsions. The solidifying points of these blends are 78° and 73° C., respectively. Wax no. 2 is easier to keep liquefied in a Waring Blendor, but wax no. 1 appears to give slightly better results under high temperature conditions, and, therefore, it is recommended for use in cream emulsions for application at high temperatures.

Cetyl alcohol appeared to make the emulsions more stable when present in small amounts. Sorbitol was added to the emulsions as a nontoxic plasticizing agent to keep the polyvinyl alcohol or gelatin soft and capable of being stretched with the growth of the fruit. However, the authors have little

evidence thus far that the addition of such a plasticizer is of much value. Sodium bicarbonate, added to polyvinyl alcohol emulsions, keeps the emulsions alkaline, which appeared to increase the effectiveness of the indolebutyric acid, probably because it is more soluble in alkaline than in acid solutions. The pH of such emulsions is between 9 and 10.

### Fluid emulsions

In table IV are presented the formulae for two typical stable fluid emulsions which have given satisfactory results in comparison with the standard triethanolamine stearate emulsion made with lanolin. Two different emulsifying agents are given. These emulsions can stand over considerable periods of time without breakdown. They are very fluid and easy to use without undue clogging of the atomizer used for their application.

TABLE IV  
FORMULAE OF FLUID EMULSIONS

EMULSION TYPE	COMPOSITION
Standard lanolin triethanolamine stearate	0.2 gm. indolebutyric acid 1.3 gm. lanolin, hydrous 0.3 gm. triethanolamine 1.0 gm. stearic acid 100.0 ml. water
Wax blend no. 2-Pharmagel B	0.2 gm. indolebutyric acid 5.0 gm. wax blend no. 2 0.3 gm. Pharmagel B 0.1 gm. sorbitol 0.5 gm. sodium bicarbonate 100.0 ml. water
Wax blend no. 2-polyvinyl alcohol	0.2 gm. indolebutyric acid 5.0 gm. wax blend no. 2 0.5 gm. polyvinyl alcohol, RH-403 0.1 gm. sorbitol 0.5 gm. sodium bicarbonate 100.0 ml. water

### PROCEDURE FOR THE PREPARATION OF FLUID EMULSIONS

The standard emulsion given for comparison in table IV may be prepared as described by HOWLETT (1). The other two liquid or sprayable emulsions were made according to the following procedure.

Two small openings were cut near the edge of the lid of a Waring Blendor jar. Through one opening was inserted an 8-mm. glass tube connected to a two-liter Erlenmeyer flask, half filled with water. The glass tube was so arranged that it was possible to slip it up and down vertically. Care was exercised to prevent the tube from catching in the blendor blades.

The wax was carefully melted in a beaker and brought to about 110° C. The indolebutyric acid was added next, keeping it just below 120° C., the point at which the acid crystals melt.

Forty per cent. of the water that was finally to be used was placed in the blendor jar; the dry polyvinyl alcohol, sorbitol, and sodium bicarbonate



were added and mixing begun. Heat was then applied to the Erlenmeyer flask and the blender quickly brought up to 100° C. by violently boiling the water in the flask, injecting the steam into the blender jar close to the mixing blades. A thermometer inserted in the second opening in the lid can be used for checking the temperature.

When the temperature reached 100° C., the melted wax-indolebutyric acid mixture was slowly added. Mixing was done with steam for about three minutes; the steam tube was then slipped out of the blender jar; and the emulsion was mixed five minutes more. The most stable emulsions are obtained by allowing the blender to finish mixing at slowest possible speed with the lid off until the mixture has cooled to about 60° C.

The contents of the blender jar were poured into a flask which was shaken gently until all the bubbles on the surface were broken. The contents were then transferred to a graduate and made up to the indicated quantity with water.

### Cream emulsions

Several cream emulsions having the consistency of lanolin were made up with other waxes mixed with lanolin. Wax no. 2 was found to make an excellent cream when Pharmagel B or polyvinyl alcohol was used as the emulsifying agent. Such creams do not leave sticky residues.

#### PROCEDURE FOR PREPARATION OF CREAM EMULSIONS

Formulae for the creams are given in table V. In preparing the cream emulsions, the indolebutyric acid was added to the melted wax as for fluid emulsions. All the water was heated to 100° C. and placed in a pre-heated Waring Blender jar. The sodium bicarbonate and Pharmagel B or polyvinyl alcohol were then added. Next the blender jar and its contents were

TABLE V  
FORMULAE OF CREAM EMULSIONS

PASTE TYPE	COMPOSITION
Wax blend no. 1-Pharmagel B	0.2 gm. indolebutyric acid 20.0 gm. wax blend no. 1 2.0 gm. Pharmagel B 0.5 gm. sorbitol 0.5 gm. sodium bicarbonate 80.0 ml. water
Lanolin-Pharmagel B	0.2 gm. indolebutyric acid 20.0 gm. lanolin, anhydrous 2.0 gm. Pharmagel B 0.5 gm. sorbitol 0.5 gm. sodium bicarbonate 80.0 ml. water
Wax blend no. 1-polyvinyl alcohol	0.2 gm. indolebutyric acid 20.0 gm. wax blend no. 1 2.0 gm. polyvinyl alcohol, RH-403 0.5 gm. sorbitol 0.2 gm. sodium bicarbonate 80.0 ml. water

brought to 100° C. in a water bath. The melted wax mixture heated to 100° C. was then added. Mixing was done at high speed for 15 minutes with the jar thermally insulated, the temperature kept at 90° C. Care must be exercised so that the thermal insulation does not prevent the circulation of air past the motor of the blender. Since the creams cannot be measured volumetrically with ease, a steam jet which adds an unknown amount of water cannot be used. The proper amount of water is added at the beginning, and no further additions should be made. The same procedure may be used for the fluid emulsions, but it takes more time than when a steam jet is used.

### Mucilage solutions

Since indolebutyric acid converted to the sodium salt is soluble in water to the extent of several tenths of a per cent., it was thought that it might be

TABLE VI

CHARACTERISTICS OF VARIOUS MUCILAGES AND WATER-SOLUBLE SUBSTANCES FOR THE SODIUM SALT OF INDOLEBUTYRIC ACID

CARRIER	FILM FORMING PROPERTIES	REMARKS
Polyvinyl alcohol, high viscosity grade DuPont, PVA, RH-403	Tough, clear, flexible film	Good carrier, solutions clear, medium viscosity
Polyvinyl alcohol, medium viscosity grade DuPont, PVA, RH-488	Tough, clear, flexible film	Good carrier, solutions clear, medium viscosity
Polyvinyl alcohol, low viscosity grade DuPont, PVA, RH-623	Moderately tough, clear, flexible film	Very good carrier, solutions clear, low viscosity
Gum arabic	Weak, brittle film	Very good carrier, solutions clear, amber color, low viscosity
Carbowax, no. 1500 or no. 4000 (polyethylene glycol)	Soft film	Very toxic, good solvent for indolebutyric acid
Glycerine	Liquid	Very toxic, good solvent for indolebutyric acid, good plasticizer
Sorbitol	Non-film-forming	Nontoxic, poor carrier, good plasticizer

possible to eliminate the complicated preparation of emulsions by simply dissolving a concentrated alcohol solution of indolebutyric acid in an alkaline solution of a mucilage such as polyvinyl alcohol, gelatin, some of the natural gums as gum arabic, or of the polyethylene glycol sold as Carbowax by the Carbide and Carbon Chemicals Corporation. The properties of these materials are given in table VI.

Carbowax was soon eliminated, however, because it was very toxic to leaf tissues and tomato flowers. Leaves of tomato, sweet pepper, eggplant, melon, sweet potato, peach, apple, chrysanthemum, and rose were used for testing. When undiluted Carbowax was applied to leaf tissue, the tissues

were killed within four days on all the species tested. When mixed with 50 per cent. distilled water, the leaf tissues of all species were killed; 10 per cent. mixtures of Carbowax in distilled water injured the leaves of some of the species, especially sweet potato and eggplant. Mixtures containing 1 per cent. and less of Carbowax did not injure any of the leaf tissues. However, 1 per cent. mixtures severely injured tomato floral parts, and some burning occurred even at concentrations as low as 0.1 per cent. This was a disappointment because Carbowax had many interesting properties and was an excellent solvent for indolebutyric acid.

Gelatin formed solutions of high viscosity as in the case of the emulsions,

TABLE VII  
FORMULAE OF MUCILAGE SOLUTIONS

SOLUTION TYPE	COMPOSITION
Glycerine-gum arabic	0.2 gm. indolebutyric acid 3.0 gm. glycerine 3.0 gm. gum arabic 0.5 gm. sodium bicarbonate 100.0 ml. water
Ethyl alcohol-gum arabic	0.2 gm. indolebutyric acid 3.0 ml. ethyl alcohol 3.0 gm. gum arabic 0.5 gm. sorbitol 0.5 gm. sodium bicarbonate 100.0 ml. water
Ethyl alcohol-polyvinyl alcohol	0.2 gm. indolebutyric acid 2.0 gm. ethyl alcohol 1.5 gm. polyvinyl alcohol, RH-403 0.1 gm. sorbitol 0.5 gm. sodium bicarbonate 100.0 ml. water

and it also tended to mold readily. Polyvinyl alcohol of the low viscosity RH-623 grade and gum arabic appeared to be the most suitable materials.

#### PROCEDURE FOR THE PREPARATION OF MUCILAGE SOLUTIONS

In table VII are given two formulae for mucilage solutions. In preparing these, the indolebutyric acid was dissolved in the alcohol. The sodium bicarbonate and sorbitol were dissolved in half the water in a Waring Blender jar and the mucilage added. The solution was mixed at high speed until the mucilage was entirely dissolved. The solution was then heated with a steam jet to 100° C., the steam shut off, and the indolebutyric acid solution added. The remaining water was added, following cooling.

These solutions are clear and practically colorless. They are lower in viscosity than the emulsions and may be applied readily. Sufficient data are not as yet available to determine whether such solutions retain their activity during storage or whether they will prove as effective as the emulsions for use as carriers in setting fruit.

### Discussion

Early in the work, it was thought that the increased incidence of blossom-end rot occurring with fruits treated with indolebutyric acid emulsions, as compared with untreated ones, was due to the toxicity of either the lanolin or triethanolamine stearate of the standard emulsion. However, as the work progressed, such increased blossom-end rot appeared to be only partly influenced by the carrier used. It appeared to be due primarily to the indolebutyric acid and to the rapid growth induced following its application. Under the environmental conditions which prevail in greenhouses in the northern United States at the time growth regulators are of value, lanolin and triethanolamine stearate usually are not visibly injurious to the floral parts at the dilutions used in the standard emulsion.

### Summary

Formulae and methods of preparation of several stable fluid and cream emulsions employing waxes other than lanolin and emulsifying agents other than triethanolamine stearate are given. Mucilage solution formulae are also presented.

The cream emulsions do not leave sticky residues as does lanolin, and they are nontoxic to plant tissue. Tomato fruit set well when these emulsions were used as carriers for indolebutyric acid. Under conditions of high temperature where lanolin and lanolin emulsions are toxic, the materials recommended are not injurious. They also offer advantages where physiologically inert carriers are desired for materials other than growth regulating substances.

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## THE ORGANIC ACIDS OF GRAPEFRUIT JUICE<sup>1</sup>

WALTON B. SINCLAIR AND D. M. ENY

(WITH ONE FIGURE)

The total acidity of grapefruit juice is highly associated with the quality of the fruit. Many of the biochemical reactions occurring in the fruit during growth and maturity depend upon the concentrations of the various acid constituents that compose the total acidity. The rates and types of reactions in citrus fruits are highly correlated with the pH, which in turn is directly related to the free and combined acids in the juice. These factors, considered collectively as the buffer system of the fruit, are important in regulating the proper reaction in the juice. The present investigation, therefore, is concerned with the determination of the organic acid constituents in grapefruit juice and the amounts of each that exist in the free and combined forms. Certain relationships are also noted between the pH and the existing cations that are available for salt formation.

### Materials and methods

The methods of fruit sampling and the analytical procedures are the same as those used and described in similar studies on oranges and lemons (7, 8, 9). It is necessary to give only a brief listing of the procedures in this paper. Total soluble solids in the juice were determined as sucrose, with a Brix hydrometer. Using the table of STEVENS and BAIER (10), the true soluble solids were obtained by making a correction for the percentage of acid in the sample. The free-acid content of the samples was titrated on an aliquot portion of the juice with standard NaOH, with phenolphthalein as indicator. The organic acid fraction was precipitated from 80 per cent. alcohol with lead acetate and separated from the filtrate by centrifuging. The precipitate was washed with alcohol, suspended in water, and freed of lead by passing H<sub>2</sub>S through the solution. The lead sulphide was filtered off and washed with water. The filtrate and washings were combined and diluted to a known volume. The citric and malic acids were determined simultaneously, on aliquot portions of this solution by the method of PUCHER, VICKERY, and WAKEMAN (6). The amount of organic acid in the combined form was estimated by determining the alkalinity of the ash from an aliquot of juice.

### Results

#### ACID CONSTITUENTS OF GRAPEFRUIT JUICE PRECIPITATED WITH LEAD ACETATE

The organic polybasic acids of grapefruit juice were precipitated in 80 per cent. alcohol with lead acetate. An analysis of this lead-free precipitate gave the organic acids and acid salts naturally present in the juice. The

<sup>1</sup> Paper No. 535, University of California Citrus Experiment Station, Riverside, California.

TABLE I

ANALYSIS OF THE ORGANIC ACIDS OF GRAPEFRUIT JUICE

SAMPLE NO. AND LOCALITY	SAM- PLING DATE	STAGE OF FRUIT MATU- RITY	FRUIT DIAMETER	TRUE* SOLU- BLE SOLIDS	PH	ACID CONSTITUENTS PRECIPITATED BY LEAD ACETATE										COMBINED ACID (AS CITRIC) DETERMINED FROM ALKA- LINITY OF ASH	FREE ACID	
						FREE ACID (AS CITRIC) DETERMINED BY PHENOL- PHTHALEIN TITRATION		TOTAL ACID TITRATED (AS CITRIC)		CITRIC ACID (PENTABROM- ACETONE METHOD)		MALIC ACID		TOTAL ACID (CITRIC + MALIC)				
						mg./ ml.	m.c./ ml.	mg./ ml.	m.c./ ml.	mg./ ml.	m.c./ ml.	mg./ ml.	m.c./ ml.	mg./ ml.	m.c./ ml.			
Fruit samples from packing house†	July 17	Yellow	8.5	12.6	2.75	20.88	0.326	26.58	0.415	22.58	0.353	4.00	0.060	26.44	0.413	1.96	0.031	91.41
	July 19	"	8.5	12.5	2.80	21.51	0.336	26.58	0.415	23.58	0.368	3.30	0.049	26.70	0.417	2.08	0.032	91.18
	July 23	"	8.5	10.7	2.86	18.22	0.285	24.55	0.383	22.94	0.358	1.80	0.027	24.65	0.385	1.90	0.030	90.55
	July 26	"	10.0	10.3	2.80	18.48	0.289	24.90	0.389	23.48	0.367	1.62	0.024	25.03	0.391	2.02	0.032	90.14
	July 27	"	10.5	10.3	2.86	17.72	0.277	24.23	0.378	21.95	0.343	2.45	0.037	24.33	0.380	2.04	0.032	89.68
	July 31	"	10.5	11.0	2.86	17.53	0.274	23.44	0.366	21.32	0.333	2.30	0.034	23.50	0.367	1.96	0.031	89.94
	Aug. 3	"	8.0	10.8	2.87	17.34	0.271	23.28	0.364	20.27	0.317	3.46	0.052	23.62	0.369	1.71	0.027	91.02
	Aug. 7	"	10.0	10.8	2.87	18.22	0.285	24.04	0.376	22.58	0.353	1.80	0.027	24.33	0.380	1.96	0.031	90.29
Fruit samples of different stages of maturity	Aug. 20	Green	5.0	7.6	3.30	19.89	0.311	27.78	0.434	25.17	0.393	2.85	0.043	27.91	0.436	4.99	0.078	80.00
	Aug. 24	"	6.0	7.9	3.08	22.72	0.355	29.02	0.453	26.43	0.413	2.55	0.038	28.87	0.451	4.49	0.070	83.50
	Aug. 26	"	6.0	7.7	3.08	19.06	0.298	25.93	0.405	23.21	0.363	2.82	0.042	25.93	0.405	3.96	0.062	82.79
	Aug. 28	"	7.5	10.5	2.96	22.84	0.357	29.03	0.453	25.90	0.405	2.97	0.044	28.74	0.449	3.03	0.047	88.29
	Aug. 29	"	8.5	9.8	3.06	19.88	0.311	27.51	0.430	25.35	0.396	2.35	0.035	27.59	0.431	3.53	0.055	84.92
	Aug. 31	"	9.3	9.9	3.08	22.84	0.357	30.29	0.473	26.08	0.407	4.10	0.061	29.96	0.468	3.28	0.051	87.44

\* Due to the relatively high acid content of the juice, a correction was made on the hydrometer readings to give the true percentage of soluble solids (10).

† The high acid concentration and small sizes of the fruit in these samples, caused by the late season, indicate that the fruit is immature.



completion with which lead acetate precipitated the organic acids is made evident by the close agreement of the total acid values with those reported for the sum of the citric and malic acids (table I). The total acid values (as citric) represent all of the acid groups precipitated from the juice with lead acetate. That inorganic acid salts are not present in the juice in appreciable amounts is shown by the fact that the citric and malic acids account for all of the acid groups in the precipitate. These results do not agree with those of MENCHIKOVSKY and POPPER (5), who reported that the grapefruit of Palestine contained, in addition to citric and malic acids, small amounts of tartaric and oxalic acids.

The citric and malic acid contents of the juice from grapefruits of different maturities are shown in table I. It should be pointed out that these values, as determined by the method of PUCHER, VICKERY, and WAKEMAN (6), include both the free and combined acids in the form of citrates and malates. As in the lemon and the orange, the actual amount of citric acid in the juice represents the major portion of the total organic acid content of grapefruit. The decrease in concentration of the free acids with the fruit maturity is due chiefly to the citric acid content. In the samples studied, the concentration of malic acid and malate showed a variation of 100 per cent. No explanation can be given for such large variations in grapefruit juice. Large differences of this sort were not encountered in mature oranges and lemons. No evidence was obtained from these investigations which would indicate the existence of an equilibrium between the concentrations of citric and malic acids.

#### RELATION OF pH TO THE FREE AND COMBINED ACIDS IN GRAPEFRUIT JUICE

The concentration of free acids of mature grapefruit juice is between that of the lemon and the orange; and, consequently, the pH is similarly related. As in oranges, the amount of free acids (mg. per fruit) increased and reached a maximum in grapefruit during the early growth period, but the decrease in concentration of free acids (mg. per ml.) during ripening was caused evidently by the growth of the fruit and the consequent dilution of the acids present; for as the grapefruit increases in size the acids must be distributed through an increasing volume of juice.

The relation of pH to the concentration of free acid in different samples of grapefruit juice is shown in table I. In the immature fruit, the amount of combined acids varies from 12 to 20 per cent. of the total acid radicles, and while the acidity is near its maximum, the pH at this point is strongly influenced by the high salt content. In the mature fruit, the free acid varied from 17.34 to 21.51 mg. per ml.; the percentage of the total acid in the free state showed little variation. This means that the combined acids (salts of inorganic cations) did not vary enough to produce more than a slight shift in pH. The average amount of organic acid in the combined form was 1.95 mg. citric acid per ml., and this amount corresponds, on the average, to a salt content of 10 per cent. of the total acid radicles.

The pH-salt relationships of grapefruit juice are illustrated in figure 1 by comparing the titration curve of the juice with that of a pure citric acid solution. The solution used in securing the data for the citric acid curve contained 17.94 mg. of citric acid per ml.; the free acid in the grapefruit juice was 16.53 mg. per ml. The pure citric acid solution had a pH of 2.25; the grapefruit juice had an initial pH of 2.87. The initial point on the grapefruit juice curve was located on the graph at pH 2.87, which corresponded to a combined acid or salt content of 10 per cent. The percentage of acid neutralized for all points, other than the initial one, included the combined acid present in the original sample and the sodium citrate formed by the neutralization of part of the free acid. The close similarity of these two curves is indicated by the pH values of grapefruit juice, which are slightly

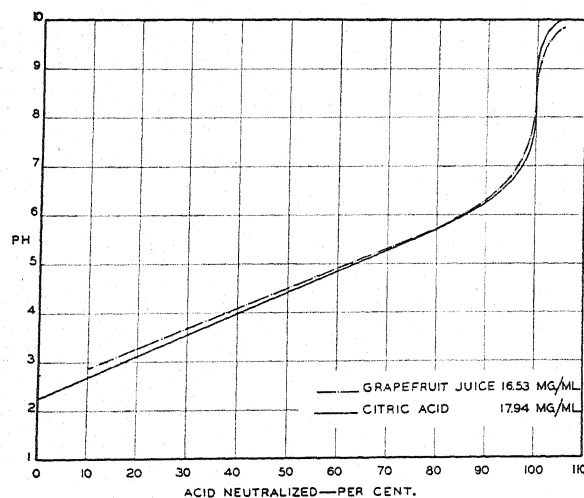


FIG. 1. Titration curves of grapefruit juice and of pure citric acid solution, showing the relation of pH to the salt content. The initial point on the grapefruit-juice curve was corrected for the salt occurring normally in the juice.

higher than those of citric acid, for a given amount of combined acid (salt). The two curves are nearly parallel and consequently have nearly the same slope. Grapefruit juice titrates like a pure citric acid solution because the free acid in the juice is composed chiefly of citric acid, and the ratio of  $\frac{\text{free acid}}{\text{combined acid}}$  is sufficiently great to diminish the salt effect on the pH. The quantity of cations that are available in the juice for combining with the organic acids is relatively small in comparison with the concentration of free acids. To plot an accurate titration curve of grapefruit juice, a correction must be made for the combined acid naturally occurring in the juice. Grapefruit juice, like all weak acids, is most effective at a pH approximating the dissociation constant of the buffer acid (pKa).

During the ripening of grapefruit, the concentration of acid in the free state decreased, and the pH of the juice increased. The rise in pH was the

result of the change in the value  $\log \frac{\text{salt}}{\text{acid}}$ , caused chiefly by the decrease in acid concentration. The combined acid reached a near constant value in the mature fruit. The difference in pH of two given samples of grapefruit juice usually amounted to the difference in the  $\log \frac{\text{salt}}{\text{acid}}$  values of the two samples. Similar results were obtained with the calculations made from the data of SINCLAIR and RAMSEY (9), which showed that juices from a green and mature orange-fruit sample, picked 7 months apart, had a difference in pH of 0.3. This change in pH was almost entirely accounted for by the corresponding difference (0.2838) in the  $\log \frac{\text{salt}}{\text{acid}}$  values of the two samples. These experimental results are theoretically derived from the equation which relates the pH to the dissociation exponent (pKa) and the ratio of salt to free acid.

THE ALKALINITY OF THE ASH AS A MEASURE OF THE COMBINED ACIDS  
AND THE BASE-FORMING ELEMENTS OF THE JUICE

The combined acids recorded in tables I to III were determined from the alkalinity of the juice ash. During the ashing process, the organic radicles are burned off leaving the equivalent cations as carbonates and oxides and some sulphates and phosphates. This alkalinity is a fair measure of the amount of cations combined with the organic acids in the juice. The concentration of combined acids (table II), as determined by this method, is greater in immature than in mature grapefruit juice. Juices of mature lemons and grapefruit have approximately the same concentration of combined acids. In mature orange juice, the concentration of combined acids is significantly greater. The specific cations combined with the organic acids have not been determined. It is highly probable, however, that the citrates (salts) are present in the juice as the potassium acid citrates, since the potassium concentration accounts for 60 to 70 per cent. of the total cations.

TABLE II  
THE COMBINED ORGANIC ACIDS IN CITRUS JUICES

STAGE OF MATURITY	COMBINED ORGANIC ACIDS (AS CITRIC) IN*					
	GRAPEFRUIT		ORANGES		LEMONS	
	mg./ml.	m.e./ml.	mg./ml.	m.e./ml.	mg./ml.	m.e./ml.
Immature .....	4.49	0.070	2.75	0.043	1.83	0.028
“ .....	3.96	0.062	2.74	0.043	1.90	0.029
“ .....	3.03	0.047	2.68	0.042	1.79	0.028
“ .....	3.53	0.055	2.50	0.039	1.88	0.029
“ .....	3.28	0.051	2.75	0.043	1.86	0.029
Mature .....	1.96	0.031	2.92	0.046	2.13	0.033
“ .....	2.08	0.032	3.01	0.047	2.08	0.032
“ .....	2.02	0.031	3.07	0.048	2.13	0.033
“ .....	2.04	0.032	2.94	0.046	1.94	0.030
“ .....	1.96	0.031	2.88	0.043	2.15	0.033

\* Determined from the alkalinity of the ash.

Extensive nutritional research has shown that citrus fruits (and others, such as tomatoes, pears, peaches, apricots, etc.) contain base-forming elements, which, when eaten and digested in the human body, have the power to neutralize the acids and, consequently, raise the pH of the urine. Although these fruits are acid in reaction, the degree of acidity or basicity in the metabolic process depends upon the extent to which the organic acid radicles are oxidized in the body, setting free cations (mostly potassium) to combine with the acid residues in the urine. When the body has an excess of basic elements to be eliminated, the ammonia secretion is greatly reduced and the organic acids combine with an equivalent of fixed base. The whole is excreted as salts which at the pH of the blood are neutral and consequently exhibit slight buffer capacity. Most fruit acids, and especially citric acid, are nearly completely oxidized in the body. Citrus juices, therefore, would not exhibit an ultimate acid effect, but they would reduce the

TABLE III

THE CATIONS COMBINED WITH THE ORGANIC ACIDS IN THE JUICE OF MATURE GRAPEFRUITS AND ORANGES

FRUIT VARIETY	TOTAL CATION CONTENTS*	CATION COMBINED WITH ORGANIC ACIDS†		CATION COMBINED WITH INORGANIC ANION
	<i>m.e. 100 gm.</i>	<i>m.e. 100 gm.</i>	%	<i>m.e. 100 gm.</i>
Grapefruit .....	5.35	3.05	57.00	2.30
Valencia oranges .....	5.96	4.26	71.47	1.70
Navel oranges .....	6.06	4.42	72.94	1.64

\* The total cation content is the sum of the Ca, Mg, K, and Na, in the juice.

† Percentage of total cation.

acidity of the urine because of the alkaline ash produced during metabolism. This factor has been definitely established by the investigations of BLATHERWICK and LONG (1). The rapid rate at which citric acid is metabolized was demonstrated by KUYPER (4) in experiments which showed that the alkali formed from digested sodium citrate was excreted in the urine at approximately the same rate as that of sodium bicarbonate. From these considerations, the deduction can be drawn that the alkalinity of the ash is a measure of the base-forming capacity of the juice, as well as a measure of the cations combined with the organic acids.

It can be seen from table II that the base-forming capacity of mature grapefruit and lemon juices is equivalent to approximately 0.032 milliequivalents per ml.; for orange juice, the base-forming capacity is greater and amounts to approximately 0.046 milliequivalents per ml. For 100 grams of juice, the base-forming capacity of Valencias, navels, and grapefruit amounted to 4.26, 4.42, and 3.05 milliequivalents, respectively (table III).

The fraction of the total cations in the combined form in mature grapefruit juice is compared, in table III, with similar values for Valencia and navel oranges. The total cation content of the different juices is represented

by the sum of the calcium, magnesium, potassium, and sodium values expressed in milliequivalents per 100 grams of juice. Undoubtedly, other cations are present in the juice in micro-quantities which would produce only a slight effect on these values. Grapefruit juices had 57.00 per cent. of their total cations combined with the organic acids, and Valencia and navel orange juices had 71.47 and 72.94 per cent., respectively. The remainder of the cations are combined in the juices with the inorganic anions, which include sulphates, chlorides, nitrates, and part of the phosphates. The titration curve of phosphoric acid demonstrates that approximately 34 per cent. is in the salt form ( $\text{KH}_2\text{PO}_4$ ) at pH 4.60. Since mature grapefruit and orange juices have a pH 3.00 to 3.70, approximately 25 per cent. of the total phosphorus is in the salt form. Lemon juice, which has a pH of 2.1, has from 12 to 15 per cent. of the phosphorus existing as a salt.

It is apparent that the amount of organic acids that combine with the mineral bases to form salts depends upon the concentration of available cations. The amount of free organic acids available for salt formation in the juice would never be a limiting factor, for the acid concentration at all times would be many times greater than the total cation content. Although the total cation content of a given sample is derived from the ash, the concentration in the juice is correlated with the mineral elements absorbed by the roots during fruit development. Soil conditions favorable for increased absorption of minerals by the plant could produce an increase in the total ash of the fruit, thereby increasing the alkalinity of the ash in the juice. The total ash content of the fruit can apparently be increased only to a limited extent by differences in cultural conditions. The total mineral constituents in vegetative portions of most plants usually respond to climatic and soil changes more readily than do those in the fruit. It can be seen from the titration curve of grapefruit juice (fig. 1) that a 100 per cent. increase in the total cation content in the juice would increase the combined acid only 10 per cent. This increase in combined acids is based, of course, on the supposition that the alkalinity of the ash increased in proportion to that of normal juice. A 10 per cent. decrease in free acid of mature grapefruit juice would cause an increase of only 0.3 of a pH (table I).

It is fully realized that the rate of formation of the free acid in grapefruit and oranges is greatly reduced by various sprays such as arsenates (3) and phosphates (2). Those sprays are most effective when applied to the trees at the early stage of acid accumulation before the free-acid concentration has reached a maximum in the fruit. Since the decrease in concentration during the maturation period is relative to the increase in volume, it would be interesting to study the effect of sprays applied at this period.

### Summary

The organic acids of grapefruit juice are citric and malic acids. The total acid content precipitated from grapefruit juice by lead acetate is equal to the sum of the citric and malic acids. The titration curve of grapefruit

juice is very similar to that of a pure citric acid solution, provided a correction is made for the combined acid naturally occurring in the juice. There is a definite relation between the pH and the amount of acid in the salt or combined form. The small fluctuations in pH of mature grapefruit juice are correlated with the large ratio of free acid/combined acid.

The free acids decreased and the pH of the juice increased with increase in fruit size. The decrease of the free-acid concentration in grapefruit during development is due chiefly to the increase in fruit size rather than to a change in the absolute amount of free acid per fruit.

The alkalinity of the ash is a measure of the base-forming capacity of the juice, as well as a measure of the cations combined with the organic acids. In grapefruit juice, more than 50 per cent. of the total cations are utilized in the formation of the organic acid salts, as compared to more than 70 per cent. in the juice of Valencia and navel oranges. A large increase in available cations from soil fertilization would produce a limited increase in combined acid and little decrease in juice acidity.

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## EFFECTS OF VARIOUS AGENTS ON THE STRUCTURAL VISCOSITY OF ELODEA PROTOPLASM<sup>1</sup>

HENRY T. NORTHERN

Ethylene chlorhydrin, thiourea, and the fat solvent anesthetics, when used in certain concentrations and exposures are often effective in breaking rest periods in plants (8, 13). These agents may also modify the rates of respiration and polysaccharide hydrolysis. The rates of such processes are frequently increased when plants are treated with relatively low concentrations and decreased when treated with higher ones (3, 6, 7, 8, 10, 13, 14, 17, 18, 19). It has been suggested (23) that the breaking of rest periods and accelerated rates of respiration, polysaccharide hydrolysis, imbibition, and permeability are a consequence of a dissociation (disaggregation) of protoplasmic proteins and that the retarded rates which result from longer exposures or higher concentrations are conditioned by an association (aggregation) of protoplasmic proteins.

The data presented later indicate that in certain concentrations ethylene chlorhydrin, thiourea, and the fat solvent anesthetics diminish the structural viscosity of protoplasm in leaf cells of Elodea. Decreases in structural viscosity are presumed to result from a dissociation of protoplasmic proteins (23).

Exposures to low temperatures may also influence plants by breaking rest periods (4) and altering the course and rate of development (vernalization). It will be shown that a temperature of 3° C. conditions a decrease in the structural viscosity of Elodea protoplasm.

As yet there is no general agreement as to the rôle of copper and zinc in the metabolism of plants, although it is known that in traces they accelerate one or more plant activities and in larger quantities they are toxic (2). In relatively low concentrations these metals lower protoplasmic viscosity whereas in higher concentrations they increase it.

### Materials and methods

For each experiment (tables I and II) one whorl of Elodea leaves was placed in tap water, which was used except as specified in the tables, and the adjacent whorl was placed in the experimental solution made up with tap water unless otherwise indicated. The above procedure was repeated with three other plants, thus giving a sample of four whorls of leaves for the experimental group and an equal number for the control group.

After the desired time of immersion at room temperature (approximately 21° C.), the control and experimental whorls were placed on cotton wet with water for the controls and the appropriate solution for the experimental group. After inserting the cotton wads in centrifuge tubes, the leaves were

<sup>1</sup> Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 200.

centrifuged with an acceleration of  $680 \times$  gravity for 90 seconds, an acceleration which usually displaced the chloroplasts only in cells at the base of the control leaves. In each experiment the control and experimental groups were centrifuged synchronously. Following centrifugation one leaf from

TABLE I

EFFECTS OF VARIOUS AGENTS ON THE STRUCTURAL VISCOSITY OF ELODEA PROTOPLASM

AGENT	CONCENTRATION	PERCENTAGE DIFFERENCES BETWEEN CONTROL AND EXPERIMENTAL GROUPS WHEN EXPOSED FOR			
		15 MIN.	30 MIN.	60 MIN.	120 MIN.
Ethylene chlorhydrin (anhydrous)	0.5%	.....	-36*	-36	-35
	1.0	-53	-68	-35	.....
	2.0	-72	-60	-91	-69
	5.0	+15	+12	+15	+18
	10.0	+12	+15	+12	+15
Thiourea	1.0%	-32	-3	+3	0
	2.0	-67	-62	-63	-42
	3.0	-79	-95	-87	.....
Ether (anhydrous)	2.0%	-38	-40	-24	-40
	3.0	-56	-42	-45	-52
	4.0	-80	-71	-53	.....
	6.0	+2	+13	+6	0
Ethyl alcohol	3.0%	-57	-42	-50	-52
	6.0	-55	-86	-72	-64
	12.0	-46	-14	-3	-24
	15.0	+39	+40	+42	.....
Propyl alcohol (normal)	1.75%	-85	-51	-64	-20
	3.50	-82	-94	-88	-83
	7.00	+8	+14	.....	+15
Butyl alcohol (normal)	0.75%	-67	-57	-35	-21
	1.50	-83	-84	-74	-54
	3.00	+14	.....	.....	.....
Sucrose	0.6 M	-95	-99	-97	-92
KCl	0.3 M	-84	-95	-97	.....
CuCl <sub>2</sub> · 2H <sub>2</sub> O in distilled water	0.00001 M	-3	-3	+2	-5
	0.00005	-1	-12	-26	-38
	0.0001	-8	-41	-62	-47
	0.001	-70	-60	+6	+14
	0.01	+9	+8	.....	.....
CuCl <sub>2</sub> · 2H <sub>2</sub> O in tap water	0.0001 M	-72	-65	-60	-26
	0.001	-78	-53	.....	+5
	0.01	-1	0	.....	.....
ZnSO <sub>4</sub> · 7H <sub>2</sub> O in distilled water	0.0001	-2	-3	0	-38
	0.0005	-10	-16	-28	-26
	0.001	-1	-7	-28	-47
	0.01	-17	-41	-35	+1
Temp. of 3° C.	.....	-84	-77	-85	-81

\* Minus (-) indicates a decrease in viscosity; plus (+) an increase.

each of the four whorls of the experimental and control groups was mounted in seventy per cent. alcohol. For the first trials two leaves from each whorl were mounted, but as the two leaves gave similar results in later experiments only one leaf from each whorl was used. Subsequently the approximate

percentages of cells in which the chloroplasts had been displaced (moved to the centrifugal wall) were determined. Each experiment was repeated at least once and the leaves, following centrifugation, were examined to determine if there was general agreement with the previous trial. The repetitions gave comparable results in all instances.

### Results

The data are summarized in tables I and II. Each figure in the columns represents the difference between the average percentage of displacement noted in the control group and in the corresponding experimental group. For example, the average percentage of cells in which the chloroplasts were displaced in the four water control leaves which were centrifuged at the same time as the four leaves which had been treated with 0.5 per cent. ethylene

TABLE II

EFFECTS OF VARIOUS AGENTS ON THE STRUCTURAL VISCOSITY OF *ELODEA* PROTOPLASM

AGENT	CONCENTRATION	PERCENTAGE DIFFERENCES BETWEEN CONTROL AND EXPERIMENTAL GROUPS WHEN EXPOSED FOR			
		1 MIN.	2 MIN.	5 MIN.	10 MIN.
Ethylene chlorhydrin	5.0%	- 67	- 68	- 64	+ 25
Ether	6.0%	.....	.....	- 30	.....
Ethyl alcohol	15.0%	.....	- 78	- 65	- 42
	18.0	- 54	- 45	+ 13	+ 25
Propyl alcohol	7.0%	.....	- 89	- 78	- 82
Butyl alcohol	3.0%	.....	- 93	- 30	.....
CuCl <sub>2</sub> · 2H <sub>2</sub> O in distilled water	0.01 M	.....	- 26	- 17	.....

chlorhydrin for 30 minutes was 12 per cent. whereas in the leaves treated with ethylene chlorhydrin the chloroplasts were displaced in 48 per cent. of the cells. The difference, 36 per cent., is the figure recorded in the table. Negative figures indicate that there was less displacement in the controls than in the experimental leaves and positive numbers indicate that the displacement was greater in the controls. Accordingly negative values indicate decreases in protoplasmic viscosity and positive ones increases.

The data in tables I and II disclose that ethylene chlorhydrin, thiourea, ether, ethyl alcohol, propyl alcohol, butyl alcohol, cupric chloride, and zinc sulphate lower the structural viscosity of the protoplasm when used in relatively low concentrations or relatively high concentrations for short periods of time. On the other hand, when higher concentrations were used, except with thiourea, the initial decrease was followed by an increase to at least normal and in some cases to higher than normal.

The 0.6 molal sucrose and 0.3 molal potassium chloride plasmolyzed the cells. Such plasmolysis induced a striking decrease in the viscosity of the protoplasm.

The viscosity was much less in cells maintained at 3° C. for periods ranging from 15 minutes to 2 hours than in cells maintained at room temperature, 21° C. Cells maintained and centrifuged at 3° C. invariably showed a greater percentage of chloroplast displacement than did the controls (those maintained at 21° C.), which were centrifuged at the same time.

### Discussion

Long ago HUXLEY suggested that protoplasm, wherever located, was similar in many particulars. If this be true, the living substance, irrespective of the body in which contained, may respond in like manner to applied agents.

Fat solvent anesthetics affect protoplasm in various organisms in a similar manner, decreasing the protoplasmic viscosity in certain concentrations and increasing it in higher ones (5, 11, 12, 26). Some of the concentrations and exposures used in this investigation were the same as those used by DAUGHERTY (5) in her studies of *Amoeba* protoplasm. The results, decreased viscosities, obtained by her for the cortical gel of *Amoeba* are like those obtained for *Elodea* when concentrations of 6 per cent. ethyl alcohol, 3.5 per cent. propyl alcohol, 1.5 per cent. butyl alcohol, and 2 per cent. ether were used. Daugherty did not use higher concentrations. When *Elodea* cells were treated with high concentrations an initial decrease was soon followed by an increase in viscosity, and such results are like those obtained with *Spirogyra* (20).

With respect to the effects of copper on protoplasmic viscosity, the only previous complete study, one in which several concentrations were used for different intervals, is that of ANGERER (1) who noted that with concentrations of cupric chloride less than  $10^{-3}$  molar the protoplasmic viscosity of sea urchin eggs decreased after a latent period whereas with longer time intervals the viscosity increased. Because the protoplasm of *Elodea* reacted likewise, copper probably affected these apparently unrelated protoplasms in a similar manner.

TIMMEL (25) working with *Caltha* and NORTHEN (22) with *Spirogyra* previously noted that plasmolysis decreases protoplasmic viscosity, a result in agreement with that presented in this paper.

In contrast to the similar responses of different kinds of protoplasm to fat solvent anesthetics, cupric chloride, and hypertonic solutions, the response of protoplasm to low temperature is not identical in the various organisms that have been studied or the methods of applying low temperatures have not been the same. According to HEILBRUNN (12) some types of protoplasm show a regular increase in viscosity with diminishing temperature whereas other types may exhibit a decrease in viscosity as the temperature is lowered in a given range. The protoplasm of *Spirogyra* is probably of the first type (21) whereas the cortex of *Amoeba proteus* is an example of the second type. When *Amoeba* were maintained in culture fluid the viscosity increased as the temperature was raised from 3° C. to 7° C., but with

further increase up to 33° C. the viscosity decreased (24). However, at 20° C. the viscosity was higher than at 3° C., a result which is in agreement with that obtained for Elodea.

Ethylene chlorhydrin and thiourea in appropriate concentrations decrease the structural viscosity of Elodea protoplasm (tables I and II). The data of MARCY (16), who studied the effects of ethylene chlorhydrin and thiourea on protoplasmic streaming, may be considered in accord with the data presented. She noted an acceleration of protoplasmic streaming in cells of Nitella and Elodea which had been immersed for 2 hours in 1 per cent. ethylene chlorhydrin and 1 per cent. thiourea. Such increased streaming may indicate that the viscosity of the protoplasm had been decreased.

Many of the chemicals used in this investigation not only affect protoplasm, but they also influence the rates of such processes as respiration, polysaccharide hydrolysis, imbibition, and permeability, generally conditioning increased rates when used in appropriate concentrations and decreased rates when used in higher ones (3, 6, 7, 8, 9, 10, 13, 14, 15, 17, 18, 19). In addition many of the agents are effective in breaking rest periods. It is believed (23) that the agents do not directly affect the processes mentioned but that the rates are influenced by the alterations of protoplasm, being increased when the protoplasmic viscosity is lowered and decreased when the viscosity is increased.

The decreases in structural viscosity recorded in this paper probably result from a dissociation of the protoplasmic proteins (that is, the splitting of large protein molecules into units of smaller size). NORTEN hypothesized (23) that such dissociation often conditions increases in the rates of such processes as respiration, imbibition, permeability, polysaccharide hydrolysis, and under some conditions the dissociation may be primarily responsible for the breaking of rest periods. The idea that a disaggregation of cell colloids (resulting in a greater dispersion) hastens the rates of many cellular processes is a rather old and well-established hypothesis. NORTEN's concept augments this older idea in that he recognizes that dissociation not only increases the surface, but it may also result in the liberation of enzymes from their previous combinations and in the formation of enzyme activators such as sulfhydryl groups.

### Summary

The centrifuge method was used to determine the effects of various agents on protoplasmic viscosity in cells of Elodea. Concentrations of 2 per cent. ethylene chlorhydrin, 3 per cent. thiourea, 4 per cent. ether, 6 per cent. ethyl alcohol, 3.5 per cent. propyl alcohol, 1.5 per cent. butyl alcohol, 0.0001 molal cupric chloride, and 0.001 molal zinc sulphate conditioned decreases in the structural viscosity when the exposures were two hours or less. Decreases in viscosity were presumed to result from dissociations of protoplasmic proteins. Concentrations one-half of those listed above also decreased the viscosity, while with concentrations greater than those mentioned an initial decrease in viscosity was usually followed by an increase.

Plasmolysis with 0.6 molal sucrose or 0.3 molal potassium chloride induced a decrease in viscosity.

The protoplasmic viscosity was lower at a temperature of 3° C. than it was at 21° C.

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## THE SOLUBLE NITROGEN FRACTIONS OF POTATO TUBERS; THE AMIDES<sup>1</sup>

F. C. STEWARD<sup>2</sup> AND H. E. STREET

(WITH SIX FIGURES)

Of the total nitrogen present in potato tubers, approximately two-thirds is in the form of compounds which are extractable by 70 per cent. alcohol. This paper deals with the nature of these soluble nitrogen compounds and more particularly with the amides. Although the amides are among the simplest substances in the soluble nitrogen fractions, complete account of them can only be rendered after somewhat laborious experiment. Furthermore these relatively simple substances, which are present in but small quantity in the tissue, command a degree of interest which is not overshadowed by the more complex nitrogenous compounds which are present in greater quantity in the cells. Indeed, the behavior of the amide-N fraction emerged as one of the outstanding features of the nitrogen metabolism of potato cells under conditions such that they possess a high degree of metabolic activity and are able to synthesize protein as well as to exhibit many other signs of vital activity—including a renewed ability to absorb and accumulate salts from dilute solutions.

Using the potato tuber as experimental material and a technique of controlling those external variables which determine the behavior of the cells, a first broad survey of the biochemistry of the tissue under conditions conducive to salt absorption has been made (49, 50, 51). Attention has been focused upon the inter-relations between salts, aerobic respiration, protein synthesis, and the effect on these several processes of changes in temperature, in oxygen supply, and in the nature and concentration of salts in the external solution. The synthesis of protein from the simpler nitrogen compounds is the predominant feature of the recrudescence of vital activity in these cells, and it has to be recognized that in the ultimate connection between protein synthesis, respiration, and salt absorption there must lie the clue to much that is not yet known about all these processes and, indeed, about the living system.

Hitherto the biochemical survey has relied upon indirect evidence drawn from quantitative analysis of a variety of nitrogen fractions; e.g., total nitrogen, soluble nitrogen, and the latter subdivided into amino-nitrogen and amide-nitrogen. From such data it was, however, possible to infer that the soluble nitrogen fraction of potato parenchyma contained certain amides in addition to the amino-acids which were responsible for much of the buffer capacity of the expressed sap at pH 8 to 10. Moreover, the amide groups were of two kinds: the one relatively stable, as in the case of asparagine;

<sup>1</sup> This is the fifth of a series of papers dealing with the biochemistry of salt absorption by plants.

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the other, "easily hydrolyzable" as in the case of glutamine. Therefore, to understand the course of protein synthesis and the way in which the "easily hydrolyzable amide" responded to experimental conditions the more critical identification and isolation of the "easily hydrolyzable amide" was regarded (49) as an "essential part of future work."

It is the primary purpose of this paper to establish the identity of the amides of potato tuber and to suggest such refinements as are now known to be necessary in the methods for their indirect analysis. Ultimately it may be necessary to furnish a similar account of the other alcohol-soluble nitrogen compounds of potato tuber. Thus, bit by bit, the biochemical evidence describes the molecular machinery with which the work of the cell is carried on, just as cell cytology and the investigation of protoplasm, its membranes and vacuoles, presents a picture of the milieu in which it operates.

In a study of the nitrogenous constituents of alfalfa VICKERY (56) accounted for 35 per cent. of the total amide as crystalline asparagine but was unable to isolate any glutamine. This strengthened the possibility, first envisaged by SCHULZE and BOSSHARD (47), that amides other than asparagine and glutamine might occur in plants. VICKERY (58) emphasized that it has been the experience of all who have attempted to determine the chemical nature of the amides found in plants that the yield of crystalline amides (asparagine and glutamine) fell far short of the quantity to be expected from their amide nitrogen content, and he considered it probable that other amides, particularly the amides of allied amino-acids, occur in plants. Hitherto the most complete account of total amide nitrogen in terms of crystalline asparagine and glutamine is that reported by VICKERY (59). Crystalline asparagine was obtained in quantity from tobacco leaves equivalent to 73.9 per cent. of that in an aqueous extract of the leaves and to 92.8 per cent. of that contained in the mercuric nitrate precipitate. The corresponding figures for glutamine were 42.6 and 65.7 per cent. The possibility of the presence of another amide or amide-like substance, therefore, remained open and was, in this particular instance, strengthened by the observations of VICKERY, PUCHER, LEAVENWORTH, WAKEMAN, and NOLAN (65) on the nitrogenous compounds present in the stem of the tobacco plant. The possible occurrence of amides other than asparagine and glutamine lent additional interest to the examination of the easily hydrolyzable amide fraction of the potato tuber and especially so because STEWARD and PRESTON (49) noted that the decrease in amino-N (Van Slyke) in their "hot" alcoholic extracts as compared with their "cold" alcoholic extracts, was not identical with the decrease expected if this were due to the hydrolysis of glutamine during the preparation of the "hot" extracts.

The experimental work now reported falls into three parts. The first part consists of a critical re-examination of the analytical procedures used to estimate the soluble nitrogen fractions of the potato tuber. In the second part the isolation of the crystalline amides from potato tuber is described.

The third part deals with the separation of these amides as pure substances and their final chemical identification. A fourth section summarizes present views on the rôle of acid amides in plant metabolism.

King Edward potato tubers were used for this work. The potatoes were peeled and cylinders of medulla removed along the long axis. From these cylinders discs were cut, 2 cm. in diameter and 1 to 2 mm. in thickness. The discs, previously blotted and weighed, were either used for the immediate preparation of extracts or dried. The blotted discs were dried in a vacuum oven at 80–90° C. for one hour and then at 60° C. for 24 hours. In this investigation the protracted use of living discs under experimental conditions was not in question. Therefore, the fastidious control of disc thickness, as in the salt absorption experiments (49), was not practiced.

### Analysis of the nitrogen fractions of potato tuber

#### TOTAL NITROGEN

Total nitrogen was determined by a micro-Kjeldahl-Gunning digestion with copper selenite (5) as catalyst. The ammonia was distilled in a Pregl micro-Kjeldahl distillation apparatus, absorbed in N/50 sulphuric acid and titrated as described under the determination of ammonia-N. The results so obtained were compared with those by use of the reduced iron method (41), adapted to a micro scale. Nitrate-N was determined by reduction with Devarda's alloy, using the residues from the total amide determinations. The quantity of nitrate-N present in the tubers was small, ranging from 0.03 to 0.08 mgm. N per gm. fresh tissue. The total-N by the reduced iron method exceeded that by the Kjeldahl-Gunning method by the equivalent of the nitrate-N.

#### NITROGEN EXTRACTED BY WATER

The present work is concerned primarily with the simpler nitrogen fractions and only indirectly with the protein-N moiety. In the previous biochemical survey, to which reference has been made, the protein-N content of the tuber was determined as that part of the total-N which is insoluble in alcohol, and this was checked against the determination of protein-N by the trichloroacetic acid method. It was shown that changes in the protein-N content which resulted from experimental treatments were the same in kind and almost identical in quantity whichever of these methods was used to determine protein-N. For convenience of extraction, therefore, the alcohol method was preferred.

Water, or more strictly the dilute solution resulting from the diluted cell sap, extracts 91 to 93 per cent. of the total-N of the fresh tissue but only 75 to 78 per cent. of the total-N of the dried tissue. In other words some part, presumably of the globulin, is "denatured" during drying and is rendered less soluble in dilute salt solution.

That part of the protein which is extracted from the dry tissue by water was made the subject of a comparison of various protein precipitants. The

techniques used were: rapid heating to 80° C., maintaining at that temperature for 10 minutes and then rapidly cooling, as recommended by VICKERY, PUCHER, CLARK, CHIBNALL, and WESTALL (63), and replacing the water lost during the heating as necessary; precipitation by (a) acetic acid and (b) colloidal iron as described by THOMAS (52); precipitation by trichloroacetic acid by a method based on GREENWALD (21), HILLER and VAN SLYKE (25) and THOMAS (52); precipitation by cupric hydroxide after the methods of HART and BENTLEY (23) and of BLISH (2); precipitation by alcohol added to 70 per cent. concentration.

From three concordant series of comparisons the various protein precipitants could be arranged in ascending order of the nitrogen precipitated from aqueous extracts of tissue. Expressing the nitrogen precipitated as mgm. nitrogen per gm. of fresh tissue, the order is as follows: heat coagulation (0.17 gm. N per gm.) < acetic acid (0.19 mgm. N) < 0.2 or 2.5 per cent. trichloroacetic acid (0.22 mgm. N) colloidal iron (0.25 mgm. N) < cupric hydroxide (0.27 mgm. N) < 70 per cent. alcohol (0.29 mgm. N) < tungstic acid (0.31 mgm. N).

The method of heating to 80° C. for 10 minutes was chosen as the general method of removing protein to avoid contaminating the extracts with other reagents as it had the added advantage that it caused no change in the amide content of the extracts and very little release of ammonia-N.

As shown by the nitrogen coagulated by heat, aqueous extracts of fresh tissue contain much more protein than extracts of dried tissue. The protein-N of various aqueous extracts made from fresh tissue during the course of this work ranged from 0.71 mgm. per gm. of tissue to 0.80 mgm. per gm. whereas the nitrogen content of the aqueous extracts of dried tissue ranged from 0.18 to 0.26 mgm. N per mg. of tissue. Therefore, the possibility is that even after heat coagulation the aqueous extracts of the fresh tissue still contained quantities of protein-N which would have been excluded by other precipitants, such as 70 per cent. alcohol. As, however, the amides, with which this work is primarily concerned, are sparingly soluble in 70 per cent. alcohol it was desirable to avoid this as solvent for the soluble-N and as precipitant for protein-N. As will become clear, there is no reason to believe that the possible presence of protein in the extracts after heat coagulation affects the determination of the amide fractions appreciably; although it does bear upon the fact that a quantitative account of the soluble-N fraction in terms of ammonia-, amide-, and amino-N is not possible on the aqueous extracts, and that the discrepancy is greater if the extract has been made from fresh rather than from dried tissue (table I). On the contrary it does become possible to account for the soluble-N in terms of ammonia-, amide-, and amino-N when the aqueous extracts (freed from heat coagulable protein) are purified, and it is immediately possible to give a satisfactory account of the alcohol-soluble nitrogen in this way (tables II, V) as STEWARD and PRESTON (49) showed. There is every reason, therefore, to believe that the aqueous extracts contain complex nitrogen compounds (protein or peptides) which are not coagulable by heat.

TABLE I  
SOLUBLE NITROGEN FRACTIONS OF FRESH AND DRIED DISCS FROM POTATO TUBERS AS SHOWN BY ANALYSIS OF AQUEOUS EXTRACTS  
AT INTERVALS DURING STORAGE

EXTRACTS TUBERS STOCK 2	NITROGEN PER GM. FRESH TISSUE					N IN THE EXTRACT AFTER HEAT COAGU- LATION
	AMMONIA-N	EASILY HYDROLYZABLE AMIDE-N	ASPARAGINE- AMIDE-N	TOTAL AMIDE-N	AMINO-N*	NH <sub>4</sub> + AMINO + AMIDE-N
Fresh tissue (E.36) .....	mgm.		mgm.		mgm.	mgm.
Dried tissue (E.39) .....	0.01	0.18	0.24	0.42	0.49	0.92
Fresh tissue (E.40) .....	0.05	0.16	0.27	0.43	6.65	1.72
Dried tissue (E.42) .....	0.001	0.18	0.27	0.45	0.74	1.95
Fresh tissue (E.46) .....	0.03	0.21	0.32	0.53	1.1	2.11
Dried tissue (E.47) .....	0.01	0.22	0.23	0.45	0.94	1.75
	0.05	0.28	0.26	0.54	1.25	2.04
						2.28

\* Amino-N (Van Slyke) minus 80% easily hydrolyzable amide-N.



Table I shows the effect on the various nitrogen fractions of drying the tissue *before* it was extracted with water. Heat-coagulable protein was removed before determining the other fractions. Drying before extraction increases the quantity of the soluble nitrogen fractions *extracted by water*; the relative effect is greatest on the ammonia fraction though the absolute amounts involved are small. Both "easily hydrolyzable amide" and asparagine amide fractions are but little affected by previous drying of the tissue though the true amino-fraction (Van Slyke amino-N corrected for 80 per cent. of the easily hydrolyzable amide-N) in water extracts is significantly increased by drying before extraction. Drying seems, therefore, to be accompanied by protein degradation with the release of amino-compounds soluble in water, or dilute salt solutions, but the concomitant effect of drying on the amide fractions is small.

#### EFFECT OF WASHING THE DISCS

The moisture content of different batches of fresh tissue used in various extractions ranged from 76.6 to 81.8 per cent.; that of tissue washed in running tap water for 24 hours ranged from 83 to 87.3 per cent. The total-N of the fresh tissue ranged from 0.26 to 0.33 per cent. of its fresh weight of which 66.7 per cent. was non-protein-N. Washing the discs in running tap water for 24 hours caused a loss of 18 to 20 per cent. of the non-protein-N, 70 per cent. of this loss being due to amino-N (Van Slyke). This confirms an earlier presumption, on indirect evidence, that one effect of washing the discs was to cause some loss of amino-acids from the cell sap (48a).

#### COMPARISON OF AQUEOUS AND ALCOHOLIC EXTRACTS OF POTATO TISSUE

Aqueous and alcoholic extracts of the fresh and dried material were prepared. The aqueous extracts were prepared by methods based on those of CHIBNALL (6), VICKERY, PUCHER, CLARK, CHIBNALL, and WESTALL (63), and of PUCHER and VICKERY (42). The methods used to prepare the alcoholic extracts were essentially similar, 70 per cent. alcohol being used as solvent since it was known that stronger alcohols were likely to hinder the extraction of the simpler nitrogenous substances. The methods of extraction used did not attempt to exhaust the material. In all cases the tissue-solvent mixture was adjusted to a definite volume and then an aliquot of the filtrate taken for analysis. Extraction is therefore "complete" if the concentration of the fractions concerned is the same in the aliquots as in the tissue-solvent mixture. The following methods of extraction were adopted.

**AQUEOUS EXTRACTS OF THE FRESH TISSUE.**—Twenty grams of fresh tissue, cut in discs, was pounded in a mortar and the juice poured off into a 100-ml. flask. The pounding was continued adding successive quantities of water to the crushed tissue which was finally transferred to the 100-ml. flask and the mortar washed out with water to adjust the total volume of the mixture to 100 ml. The mixture was shaken for  $\frac{1}{2}$  hour, allowed to settle, and the

upper clear liquid strained off through fine silk. This liquid was then rapidly heated to 80° C. for 10 minutes, rapidly cooled, and then filtered through paper. Five ml. of this extract were equivalent to 1 gram of fresh tissue.

**AQUEOUS EXTRACTS OF THE DRIED MATERIAL.**—A weighed amount of the dried material in no. 60 powder was placed in a 100-ml. flask, 70 ml. of water added, and the flask and contents stored at 5° C. for 18 hours, shaking occasionally. At the end of this period the flask and contents were allowed to reach room temperature, the volume of the contents adjusted to 100 ml. with water, stored for a further 2 hours at 5° C., shaking occasionally, and then rapidly filtered through paper. The clear filtrate was rapidly heated to 80° C. for 10 minutes, rapidly cooled, and then filtered.

These methods of preparing aqueous extracts from fresh and dried tissue were found to give consistent results for ammonia-N, if the proportion of tissue to extract did not exceed 0.4 gm. of fresh tissue to 1 ml. of extract. In preparing extracts for analysis STEWART and PRESTON previously used much more solvent than this per gm. of tissue.

**ALCOHOLIC EXTRACTS OF THE FRESH TISSUE.**—Twenty grams of the fresh tissue discs were covered with 48 ml. of 95 per cent. alcohol and allowed to stand for 18 hours at 5° C., shaking occasionally. The clear, yellow, supernatant liquid was poured off into a 100-ml. flask and the tissue residue pounded with 70 per cent. alcohol in a mortar. The mortar was washed and the pounded tissue transferred to the 100-ml. flask with sufficient 70 per cent. alcohol to adjust the volume of the tissue-solvent mixture to 100 ml.; the suspension was then shaken for  $\frac{1}{2}$  hour and filtered.

**ALCOHOLIC EXTRACTS OF THE DRIED MATERIAL.**—These extracts were prepared exactly as the aqueous extracts of dried material, except that 70 per cent. alcohol was used as solvent in place of water.

The alcoholic extracts, *when compared with aqueous extracts prepared at the same time from the same stock of fresh or dried material*, gave lower values for easily hydrolyzable amide, asparagine-amide, and total-soluble-N (table II). The amides were incompletely extracted by 70 per cent. alcohol at a "concentration" of 0.1 grams of fresh tissue to 1 ml. of extract. At greater proportions of tissue to extract 70 per cent. alcohol was markedly less efficient as a solvent for the amide nitrogen fractions. Furthermore, the presence of the alcohol interfered with the subsequent estimation of the easily hydrolyzable amide-N giving a low value. This effect made it at first appear that the main loss of amide-N on extraction with 70 per cent. alcohol was due to incomplete extraction of the easily hydrolyzable amide-N portion. However, even when the alcohol was removed at 40° C. in vacuo prior to the analysis of the extracts, it is seen that both amides are incompletely extracted (table II).

It is clear, therefore, that tests for complete extraction of the amides should always be made whenever cold 70 per cent. alcohol is to be adopted as a solvent for the nitrogenous constituents of plant material.

TABLE II  
NITROGEN FRACTIONS IN AQUEOUS AND ALCOHOLIC EXTRACTS OF FRESH AND DRIED DISCS FROM POTATO TUBERS

Source	Extract	Gm. fresh tissue per ml. of extract	Nitrogen per gm. fresh tissue				Amino-N*	Total non-coagulable-N
			Ammonia-N	Easily hydrolyzable amide-N	Asparagine-amide-N	Total amide-N		
Fresh tissue (Stock 2)	Aqueous	E.38	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
		E.40	0.4	0.18	0.24	0.42	0.71	1.73
	70% alcohol	E.37	0.2	0.18	0.27	0.45	0.74	1.75
		E.41	0.2	0.12	0.23	0.35	0.82	1.36
Dried tissue (Stock 1)	Aqueous	E.29	0.2	0.13	0.20	0.33	0.72	1.23
		E.30	0.2	0.26	0.39	0.65	1.3	2.99
		E.31	0.65	0.25	0.40	0.65	1.2	2.96
	70% alcohol	E.51	0.2	0.23	0.33	0.56	0.9	2.65
		E.52	0.1	0.17	0.18	0.35	.....	1.59
				0.22	0.20	0.42	.....	1.75

\* Amino-N (Van Slyke) minus 80% easily hydrolyzable amide-N.

## DETERMINATION OF AMMONIA NITROGEN

Ammonia-N was determined by distillation in vacuo at 40° C. according to the method of VICKERY, PUCHER, and LEAVENWORTH (64), using in each determination a quantity of extract equivalent to 2 gm. or more of fresh tissue. The ammonia was absorbed in 5 ml. of N/50 sulphuric acid. The excess acid was titrated against N/100 sodium hydroxide using 3 drops of Tashiro's indicator.<sup>3</sup> In each determination the ammonia was equivalent to not less than 0.2 ml. N/100 sodium hydroxide. Duplicate readings agreeing to 0.02 ml. N/100 sodium hydroxide could be obtained over the whole range (0.014–2.8 mgm. N) used in the trials. Using this method the fresh tissue was found to contain 0.001–0.016 mgm. ammonia-N per gm.; the dried material contained 0.03–0.08 mgm. ammonia-N per gm. fresh tissue. Similar values were obtained using an aeration apparatus as described by WOOLF (72). Distillation in the Pregl micro-Kjeldahl distillation apparatus gave higher values for the ammonia-N.

The buffered alkaline mixture of PUCHER, VICKERY, and LEAVENWORTH or an excess of magnesium oxide produced distillation mixtures of pH 9.68 to 9.74 and these gave, under the same distillation conditions, similar values for ammonia-N. Excess of calcium oxide gave a distillation mixture of pH about 12.3, and this increased the apparent ammonia-N content (table III).

To reveal the source of the increase of ammonia-N resulting from distillation of aqueous extracts in the micro-Kjeldahl apparatus as compared with distillation under reduced pressure at 40° C., the extracts were analyzed subsequent to the ammonia determinations. The higher values for ammonia-N were correlated with lower values for easily hydrolyzable amide and asparagine-amide-N. Although the absolute increase in ammonia-N consequent upon distillation in the micro-Kjeldahl apparatus was small it could be accounted for to the extent of 80 per cent. by a concomitant decrease in amide-N, and, as this was also associated with some loss of amino-N (Van Slyke), it was evident that the glutamine-like amide was being affected by these distillation conditions.

## DETERMINATION OF TOTAL AMIDE NITROGEN

The method used for the determination of total amide-N was based on that of VICKERY, PUCHER, and LEAVENWORTH (64). The hydrolysis was conducted in the hydrolyzing tubes described later under the method for "easily hydrolyzable amide-N." The ammonia was recovered by distillation at 40° C. in vacuo as described in the previous section.

The content of asparagine amide-N was determined as the difference between total amide-N and the "easily hydrolyzable amide-N." The content of asparagine amide-N so calculated will represent the free asparagine present in the tissues provided that no polypeptides, containing amide groups, are present in the extracts and suffer hydrolysis under the conditions employed.

<sup>3</sup> One hundred ml. of 0.02 per cent. methyl red in 50 per cent. alcohol plus 15 ml. of 0.1 per cent. methylene blue in water.

TABLE III  
COMPARISON OF VARIOUS PROCEDURES FOR THE DETERMINATION OF AMMONIA  
(AMMONIA-N AS MGM. PER GM. FRESH TISSUE)

METHOD OF AMMONIA RECOVERY	DISTILLATION FOR 10 MINUTES IN MICRO-KJELDAHL APPARATUS			DISTILLATION FOR 10 MINUTES AT 40° IN VACUO			AERATION
	MAGNESIUM OXIDE	BUFFERED ALKALINE MIXTURE	MAGNESIUM OXIDE	MAGNESIUM OXIDE	CALCIUM HYDROXIDE	BUFFERED ALKALINE MIXTURE	
E.53	m gm. 0.072	m gm. 0.101	m gm. 0.050	m gm. 0.057	m gm. 0.050	m gm. 0.048	m gm. 0.048
E.54	0.07	0.087	0.042	0.049	0.041	0.040	0.040
E.31	0.069	0.088	0.038	0.042	0.037	0.037	0.037
E.33	.....	.....	0.048	0.051	0.047	0.045	0.045

The tissue contained from 0.42 to 0.66 mgm. total amide-N per gram fresh tissue and 0.23 to 0.40 mgm. asparagine amide-N per gram of fresh tissue.

#### DETERMINATION OF EASILY HYDROLYZABLE AMIDE NITROGEN

The tissue extracts were submitted to the mild hydrolysis employed by VICKERY, PUCHER, CLARK, CHIBNALL, and WESTALL (63) for the estimation of glutamine. The hydrolysis was performed in 25 × 200 mm. Pyrex thick-walled test tubes, closed with a rubber stopper carrying an air condenser constructed of 30 inches of glass tubing of 4 mm. internal diameter. The ammonia was recovered by vacuum distillation as described above under the determination of ammonia nitrogen.

The fresh tissue contained 0.15–0.28 mgm. easily hydrolyzable amide-N per gram of fresh tissue (average value 0.23 mgm. N per gram fresh tissue).

This standard method for the determination of easily hydrolyzable amide-N was modified in various ways to see how far the conditions of hydrolysis of the "easily hydrolyzable amides" of potato tissue were identical with those of glutamine.

After 2 hours' hydrolysis at pH 6.5, 99.7 per cent. hydrolysis of glutamine has occurred, and hydrolysis was complete in 2 hours; i.e., the increase in glutamine amide-N on prolonging the hydrolysis from 2 to 3 hours was only 0.3 per cent. A glutamine-asparagine solution was prepared with an amide content comparable to that of the potato extracts. If the hydrolysis of this solution was prolonged from 2 to 3 and 4 hours, the increases obtained were 1.4 and 3.0 per cent., respectively. Crystalline mixtures (45.III) were selected because they contained the easily hydrolyzable amides and asparagine-amide in similar proportions to that in the tissue. The yield of easily hydrolyzable amide-N was increased by prolonging the hydrolysis from 2.0 to 3.0 and 4.0 hours by only 2.0 and 3.8 per cent., respectively. Solutions obtained by liberating the nitrogen compounds from mercuric nitrate precipitates responded to hydrolysis prolonged from 2.0 to 3.0 hours by an increase of 2.9 and 4.0 per cent., respectively, in the yield of easily hydrolyzable amide-N. The easily hydrolyzable amide nitrogen of various aqueous extracts of potato tuber tissue increased with the period of hydrolysis from two hours to three and four hours by increases of 6.0 and 8.5 per cent., respectively, in the amide as so determined. Clearly the more purified the extracts become, the more closely does the time required to hydrolyze the easily hydrolyzable amide resemble that expected from glutamine in contact only with asparagine.

The hydrolysis of the easily hydrolyzable amides of potato tissue and of glutamine were both retarded by alcohol to a similar extent.

If the concentration of alcohol in the hydrolyzing solution was about 23 per cent. (as when using extracts containing 70 per cent. alcohol) the hydrolysis after 2 hours' heating had proceeded to the extent of 86 per cent. in the case of a solution of pure glutamine; whereas for the easily hydrolyzable amides in the tissue extracts it had proceeded to the extent of 71 to 80 per cent.



Therefore, for the analysis of amides the alcohol must be removed from the extracts, under conditions which do not cause decomposition of the unstable amide fraction; otherwise low values for easily hydrolyzable amide-N and correspondingly high values for asparagine amide-N will be obtained.

#### DETERMINATION OF AMINO NITROGEN

Amino-N was determined in the Van Slyke micro-amino-apparatus (53, 54) using a reaction time of 10 minutes. Extract yielding 0.7–2.0 cc. of nitrogen gas, freed from ammonia if necessary and acidified with acetic acid, was used for each test. If undue frothing occurred when the reaction chamber was shaken, 1 drop of toluene was added to the extract before it was drawn into the reaction-chamber. As recommended by the Committee on Methods of Analysis of the American Society of Plant Physiologists (1), alcohol was removed from the alcoholic extracts by distillation in vacuo at 40° C. prior to the determination of the amino-N. The value for amino-N obtained by this method is referred to as "amino-N (Van Slyke)."

When the amino-N (Van Slyke) of a solution of glutamine was determined, it was found that 91.9 per cent. of its total-N was evolved as nitrogen gas in 10 minutes. This corresponded to the conversion to nitrogen of 84 per cent. of the amide group. When glutamine is submitted to hydrolysis at pH 6.5, under the conditions here used for the determination of easily hydrolyzable amide-N, it yields quantitatively ammonia and pyrrolidone-carboxylic acid, so that complete loss of amino-N (Van Slyke) occurs (42, 63). Therefore, upon hydrolysis of glutamine at pH 6.5, and as shown by VICKERY, CHIBNALL *et al.* (63), the fraction

#### decrease in amino-N (Van Slyke) as a result of amide hydrolysis easily hydrolyzable amide-N content

has a value of 1.8. This value should be obtained with plant extracts containing glutamine, provided that no other substances are present which produce ammonia or change in amino-N (Van Slyke) content under the conditions of glutamine hydrolysis. Lower values than 1.8 would be expected with compounds such as allantoin and urea (39, 63) or unstable peptides of the type prepared by MELVILLE (34), which resemble glutamine in their stability and give, though not so markedly, high values in the Van Slyke amino-N determination. Thus, in presence of glutamine, the true amino-N calculated as amino-N (Van Slyke) minus 80 per cent. glutamine amide-N.

The decrease in amino-N (Van Slyke) under the conditions of glutamine hydrolysis is additional qualitative evidence of the presence of this amide and serves to distinguish it from other substances such as urea and allantoin which give rise to ammonia on mild hydrolysis (8, 63). In the cases examined the ratio varied from 1.47 to 1.77 (table IV). This result is consistent with the identity of the easily hydrolyzable amide with glutamine although in the work of STEWARD and PRESTON (49), the amino-groups seemed too

stable under conditions of amide hydrolysis for this hypothesis to be accepted. In the earlier work amide-N was determined by the increase in ammonia-N in "hot" as compared with "cold" alcohol extracts, and it now seems probable that under these conditions of amide hydrolysis the glutamine yielded glutamic acid, wholly or in part, rather than pyrrolidone carboxylic acid.

The amino-N (Van Slyke) value was normally determined on extracts from which ammonia had been removed under conditions which did not cause hydrolysis of the "easily hydrolyzable amides." If, therefore, the easily hydrolyzable amide fractions consist entirely or mainly of glutamine then the amino-N (Van Slyke) value will exceed the true amino-N by an amount equal to 80 per cent. of the easily hydrolyzable amide-N.

Table V shows a group of analyses in which the sum of the amino-N (Van Slyke), total amide-N, and ammonia-N, and this sum minus 80 per

TABLE IV

THE EFFECT OF AMIDE HYDROLYSIS ON THE VALUE FOR AMINO-N (VAN SLYKE)

EXTRACT	NITROGEN PER GM. FRESH TISSUE				LOSS OF AMINO-N (VAN SLYKE) AS A FRACTION OF EASILY HYDROLYZABLE AMIDE-N
	EASILY HYDROLYZABLE AMIDE-N	AMINO-N (VAN SLYKE)			
		AFTER REMOVAL OF AMMONIA-N	AFTER HYDROLYSIS OF THE EASILY HYDROLYZABLE AMIDES	AFTER HYDROLYSIS OF THE TOTAL AMIDES	
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
E.29	0.26	1.54	1.07	1.29	1.69
E.30	0.25	1.22	0.85	1.2	1.48
E.42	0.21	1.27	0.93	.....	1.60
E.53	0.28	1.51	0.01	1.13	1.77
E.54	0.23	1.31	0.97	1.15	1.47

cent. of the easily hydrolyzable amide-N, is expressed as a percentage of the total-N in the extracts after removing any heat coagulum. The sum of amino-N (Van Slyke), total amide-N, and ammonia-N does not account fully for the total soluble-N of the aqueous extracts but tends to exceed it in the purer extracts, the excess increasing in the series: purified aqueous extracts < alcoholic extracts < the crystalline mixtures in ascending order of purity. (These mixtures were obtained in the work on the isolation of the amides later described herein.) When, however, the value for amino-N (Van Slyke) is reduced by an amount equal to 80 per cent. of the easily hydrolyzable amide-N the sum of *true* amino, amide, and ammonia-N expressed as a percentage of the total non-protein-N increases through the series: aqueous extracts < purified aqueous extracts < alcohol extracts < crystallizations from the original mother-liquor < re-crystallized mixtures. The total of the soluble nitrogen fractions thus analyzed never significantly exceeded the total soluble-N of the extracts although it gradually approached this as purification proceeded until, in the purest crystalline mix-

tures examined, an almost exact account of the total-N was rendered. The account, by the means described, of the soluble nitrogen of the alcoholic extracts of the tissue tended to be 7 per cent. too low (average of 3), or ignoring one low result, only about 2 per cent. too low. STEWARD and PRESTON (49) obtained evidence that the amide group of the easily hydrolyzable amide fraction reacted to the extent of 79–92 per cent. in the Van Slyke determination. The evidence here presented amply confirms the reactivity of this amide group in the Van Slyke determination and shows that the behavior of the tissue extracts is in close accord with that of a pure solution of glutamine in this respect; i.e., 80 per cent. of amide group reacts in Van Slyke determination.

The true amino-N is, therefore, best estimated by correcting the Van Slyke amino-N on this basis. The true amino-N (amino-N (Van Slyke)—80 per cent. easily hydrolyzable amide-N) for all the samples of fresh tuber tissue which were examined fell within the limits 0.7 to 1.05 mgm. amino-N per gm. The dried tissue contained 1.1 to 1.35 mgm. true amino-N per gm. fresh tissue. The true amino-N, as thus defined, includes the amino-N groups of the amino acids of both stable and easily hydrolyzable amide fractions.

If the amino-N (Van Slyke) was determined on aqueous extracts after the acid hydrolysis used in the determination of total amide-N, the values so obtained were lower than those obtained on the original extracts freed from ammonia, but they were higher than the results obtained after the mild hydrolysis used in the determination of the easily hydrolyzable amide-N (table V). This may be due to hydrolysis of peptides during the total amide determination or, if the easily hydrolyzable amides are glutamine, to inhibition of pyrrolidone carboxylic acid formation in strongly acid solution (18).

#### Isolation of crystalline amides from potato tuber

Present methods for the isolation of acid amides from plant tissues are based on the pioneer researches of E. SCHULZE and his co-workers. SCHULZE employed lead acetate to clarify the plant extract and mercuric nitrate (45) to precipitate the amides. NEUBERG and KERB (37) in 1912 used mercuric acetate as an alternative precipitant.

Glutamine was isolated by SCHULZE and BOSSHARD (46) in 1883 by treating the fresh sap of the beetroot with lead acetate and then treating the filtrate with a neutral solution of mercuric nitrate. VICKERY, PUCHER, and CLARK (61), using the same method, obtained a quantitative yield of glutamine. EISENCHIMMEL (16) employed the NEUBERG and KERB reagent (37) to isolate glutamine from beetroot juice.

#### PRECIPITATION OF NITROGEN FRACTIONS OF POTATO EXTRACTS WITH VARIOUS REAGENTS

Fractionations by procedures based on those of VICKERY, PUCHER, and CLARK (61) and of VICKERY (57) were carried out to develop a method suit-

TABLE V  
NITROGEN IN EXTRACTS IN TERMS OF AMMONIA-N, AMIDE-N, AND AMINO-N

	NITROGEN PER GM. FRESH TISSUE					SUM OF AMMONIA-, AMIDE-, AND AMINO-N (VAN SLIKE) AS PERCENTAGE OF THE TOTAL-N IN EXTRACTS*	SUM OF THE AMMONIA-, AMIDE-, AND AMINO-N (VAN SLIKE) MINUS 80% OF THE EASILY HYDROLYZABLE AMIDE-N AS A PERCENTAGE OF THE TOTAL-N IN EXTRACTS*
	AMMONIA-N	EASILY HYDROLYZABLE AMIDE-N	TOTAL AMIDE-N	AMINO-N (VAN SLIKE)	TOTAL-N IN EXTRACTS		
	mgm.	mgm.	mgm.	mgm.	mgm.	%	%
Aqueous extracts of fresh tissue							
E.34 .....	0.016	0.26	0.60	1.22	2.30	80	71
E.40 .....	0.001	0.18	0.45	0.88	1.75	76	68
E.46 .....	0.009	0.22	0.45	1.12	2.04	77	69
Aqueous extracts of dried tissue							
E.29 .....	0.04	0.26	0.65	1.54	2.79	80	72
E.32 .....	0.04	0.23	0.61	1.36	2.50	80	73
E.42 .....	0.03	0.21	0.53	1.27	2.11	87	79
E.47 .....	0.05	0.28	0.54	1.47	2.28	90	81
Purified aqueous extracts							
E.31.D .....	0.04	0.21	0.48	0.97	1.61	92	83
E.32.D .....	0.016	0.20	0.45	1.03	1.72	87	77
E.32.E .....	0.001	0.19	0.41	0.88	1.26	102	90
E.45.D .....	0.49†	0.18	0.35	0.84	1.68	100	91
E.46.D .....	0.35†	0.16	0.37	0.74	1.46	100	93
70% alcohol extracts							
E.41 .....	0.01	0.16	0.37	0.96	1.23	109	99
E.43 .....	0.02	0.14	0.29	0.83	1.23	93	84
E.44 .....	0.02	0.17	0.35	1.0	1.31	104	95

\* After removal of heat coagulum, if any.  
† Neutralized with ammonia.

TABLE V—(Continued)

	NITROGEN PER 100 MGAL. CRYSTALLINE MATERIAL					SUM OF AMMONIA, AMIDE-, AND AMINO-N (VAN SLIKE) AS PERCENTAGE OF THE TOTAL-N IN EXTRACTS*	SUM OF THE AMMONIA, AMIDE-, AND AMINO-N (VAN SLIKE) MINUS 80% OF THE EASILY HYDROLYZABLE AMIDE-N AS PERCENTAGE OF THE TOTAL-N IN EXTRACTS*
	AMMONIA-N	EASILY HYDROLYZABLE AMIDE-N	TOTAL AMIDE-N	AMINO-N (VAN SLIKE)	TOTAL-N IN EXTRACTS		
	mgm.	mgm.	mgm.	mgm.	mgm.	%	%
First crystallizations from mother liquor							
46.iii	.....	1.58	8.26	10.5	18.38	102	95
46.iv	.....	5.11	8.19	12.7	18.53	113	90
46.v	0.29	3.74	7.01	10.8	16.9	107	89
46.vi	0.64	4.61	5.58	11.4	14.9	118	93
46.viii	0.42	3.70	5.84	11.0	15.85	109	91
Recrystallized material							
45.iii.1	.....	3.65	7.7	12.3	17.12	117	100
45.iii.3	.....	6.08	7.84	13.1	16.7	125	96
45.iii.3a	.....	6.17	8.1	12.9	17.3	121	99
45.iii.3b	.....	5.5	7.54	12.6	16.6	127	100
45.iii.1a	.....	3.75	5.54	10.95	13.46	122	100
45.iii.1b	.....	4.91	7.69	14.9	19.3	117	97
45.iii.1c	.....	2.73	8.54	11.4	17.7	113	100

able for the isolation of the easily hydrolyzable amides and to see how far they behaved like glutamine to the precipitant used. These experiments showed (table VI):

1. The easily hydrolyzable amide-N survives lead acetate precipitation and is almost quantitatively removed by mercuric nitrate reagent; and on transfer from the mercuric nitrate precipitate, it largely escapes subsequent precipitation with phosphotungstic acid. The yield of this fraction as precipitated by the Neuberg and Kerb reagent is not as good as by mercuric nitrate.

TABLE VI

PERCENTAGE RECOVERY OF THE VARIOUS NITROGEN FRACTIONS IN PURIFIED EXTRACTS OF POTATO TUBER

NITROGEN FRACTION IN THE EXTRACT	RECOVERY OF THE NITROGEN FRACTIONS: NITROGEN FRACTION IN THE PURIFIED EXTRACT AS PER- CENTAGE OF THAT SAME FRACTION IN THE ORIGINAL AQUEOUS EXTRACT OF THE TUBERS				
	EXTRACT CLARIFIED BY TREATMENT WITH EXCESS LEAD ACETATE REAGENT		EXTRACTS PREPARED BY DECOMPOSITION OF THE MERCURY COMPOUNDS PRE- CIPITATED FROM AQUEOUS EXTRACTS CLEARED BY LEAD ACETATE		EXTRACTS PRE- PARED FROM PHOSPHOTUNGSTIC ACID PRECIPITATES FROM EXTRACTS OBTAINED FROM PRECIPITATED MERCURY COMPOUNDS
	E.31	E.32	MERCURIC NITRATE REAGENT E.31	NEUBERG AND KERB REAGENT E.32	
	%	%	%	%	%
Ammonia-N .....	100	112	100	40	2.5
Easily hydrolyzable amide-N ..	92	97	89	86	81
Asparagine-amide-N .....	93	87.5	85	73	65
Amino-N* .....	97	96	91.5	83	62
Sum of ammonia-, amide-, and amino-N .....	95	95	90	80	64
Total soluble-N extracted† .....	93	93	65	69	50

\* Van Slyke amino-N minus 80% of amide-N.

† Excluding heat coagulum.

2. The asparagine amide-N survives lead acetate precipitation, is almost quantitatively removed by mercuric nitrate (but less so by Neuberg and Kerb reagent), and is much more liable to reprecipitation by phosphotungstic acid.

3. The true amino-N of aqueous extracts, which also survives lead acetate precipitation, is removed almost quantitatively by mercuric nitrate (less so by Neuberg and Kerb's reagent) and is reprecipitated by phosphotungstic acid to approximately the same degree as the asparagine amide-N.

4. That part of the total-N of the aqueous extracts for which no account has yet been rendered up to this point (nitrogen other than heat-coagulated



nitrogen and ammonia-, amino-, and amide-N) escapes lead acetate precipitation and is precipitated less readily by mercuric nitrate and more readily by phosphotungstic acid than the other nitrogen fractions. While the behavior toward lead acetate rather precludes this being stable protein it must be some more complex fraction than the others and is probably rich in diamino-acids; this warrants further investigation beyond the scope of this paper. Therefore the basis of the quantitative isolation of the mixed amides of potato was:

1. Precipitation of aqueous extract by lead acetate.
2. Precipitation by mercuric nitrate from the lead-free filtrate.
3. Crystallization from the solution obtained on liberation of the nitrogen fractions from the mercury precipitate.

The product so obtained must contain both asparagine and easily hydrolyzable amide as well as some amino-N other than that accounted for by asparagine or glutamine. From this point the isolation of pure products depends on fractional crystallization. This procedure was put into effect on a relatively large scale as follows.

#### FRACTIONATION OF THE NITROGEN IN THE EXPRESSED SAP OF POTATO TUBERS (LARGE SCALE)

The expressed juice was fractionated as described below. Prior to expression of the juice the tubers were washed, peeled, wiped with a dry cloth, cut into thin discs which were blotted, and immediately weighed. Two batches of 2.5 kgm. (extract 45) and 3.6 kgm. (extract 46) were used. The sequence of operations as indicated by the treatment of 3.6 kgm. in preparation of E.46 was as follows.

PREPARATION OF EXTRACT "A".—Three and six-tenths kgm. of the fresh tubers were minced and the sap expressed in a double-action tincture-press. From the minced marc a second portion of sap was expressed and a third portion after remincing the marc with washed, dried, silver sand. The total yield was 2050 ml. and since the moisture content of the original fresh tissue was 81.3 per cent. of the fresh weight, the sap so expressed was equivalent to the nitrogen fractions of 2521 gm. of tissue. Fifty ml. of this liquid was removed for the analysis of extract A.

PREPARATION OF EXTRACT "B".—The remainder of extract A was immediately heated to 75° C. for 10 minutes, rapidly cooled, and 2140 ml. of clear filtrate were collected; 60 ml. were removed for the analysis of Extract B.

PREPARATION OF EXTRACT "C".—The remainder of extract B was treated with a slight excess (275 ml.) of lead acetate reagent. The precipitate was removed by filtration, washed on the filter with boiled and cooled distilled water, and then rejected. The combined filtrate and washings (2275 ml.) constituted extract C, of which 50 ml. was removed for analysis. The aliquot of extract C removed for analysis was treated with hydrogen sulphide, precipitated lead sulphide removed by filtration, and the filtrate and

washings freed from hydrogen sulphide by distillation at 40° C. in vacuo. The residue was diluted to 50 ml. with water and was ready for the analysis of extract C.

PREPARATION OF EXTRACT "D".—The remainder of extract C was treated with a slight excess (250 ml.) of mercuric nitrate reagent, neutralized to litmus by addition of 20 per cent. w/v solution of sodium hydroxide and set aside overnight in the refrigerator. A white precipitate settled, leaving a clear supernatant liquor which was decanted and passed through the filter. The precipitate was suspended in 500 ml. of distilled water, shaken and allowed to stand, and the supernatant liquor again removed. The precipitate collected on a hard paper was washed with distilled water, the washings being rejected. To the mercuric nitrate precipitate suspended in 1000 ml. of distilled water 1 ml. of 1/N sulphuric acid was added and the suspension treated with hydrogen sulphide. After removal of the filtered and washed mercuric sulphide the combined filtrate and washings were freed from excess hydrogen sulphide by distillation at 40° C. in vacuo for 20 minutes. The solution was then neutralized to litmus with dilute ammonia (14 ml.) From the resultant neutral solution (1300 ml.) 50 ml. was removed for analysis of extract D.

PREPARATION OF CRYSTALLINE PRODUCTS FROM EXTRACT "D".—The remainder (1250 ml.) of extract D was concentrated by distillation to 400 ml. and freed from the last traces of mercuric sulphide by filtration through paper pulp. The combined filtrate and washings were then reduced to about 190 ml. and set aside overnight in the refrigerator. The crystalline deposit which separated (46 I.) was collected and washed with 5 ml. of boiled, cooled, distilled water. After filtrate and washings were again concentrated to about 125 ml., a further crystalline deposit (46.III) separated overnight in the refrigerator, and this was treated as described for 46.I. On concentrating the remaining solution to 60 ml. the crystalline deposit (46.III) separated. This was collected and washed with 10 ml. of 70 per cent. alcohol. From this point the crystals were washed with alcohol, hot water, and the further crystallization was carried out progressively from richer mixtures of alcohol. The filtrate and washings from 46.III were reduced to 40 ml.; crystalline deposit (46.IV) was separated overnight and washed with 10 ml. of 70 per cent. alcohol. The filtrate and washings from 46.IV were reduced to 30 ml., and 50 ml. of 80 per cent. alcohol was added with constant stirring. From the liquor the crystalline deposit (46.V) separated, and this was in turn collected and washed with 70 per cent. alcohol. To the filtrate and washings (70 ml.) was added an equal volume of 90 per cent. alcohol and on setting aside the liquid in the refrigerator crystalline deposit (46.VI) separated; this was collected and washed with 80 per cent. alcohol. The mother liquor, set aside for several days in the refrigerator, gave a further deposit (46.VII). All these crystalline deposits (46.I to 46.VII) were collected on Whatman no. 50 paper, dried in a vacuum desiccator over sulphuric acid for at least 12 hours, and weighed.

For the larger vacuum distillations referred to above a distillation apparatus similar to that described by VICKERY and PUCHER (60) was used. Concentration of the mother liquors was effected by vacuum distillation on a water bath at a temperature of 40° C.

The analysis of the nitrogen fractions of the various extracts and crystalline deposits is shown in table VII, and the yield of the various nitrogen

TABLE VII

NITROGEN ANALYSES SHOWING THE COMPOSITION OF THE EXTRACTS EQUIVALENT TO 2500 GM. OF FRESH TUBERS (EXTRACT NO. 46) AND OF THE CRYSTALLINE DEPOSITS OBTAINED (46.i-vii) DURING PROGRESSIVE PURIFICATION OF AMIDE FRACTIONS

EXTRACT 46	NITROGEN PER GM. OF FRESH TISSUE					TOTAL SOLIDS. MGM. PER GM. OF FRESH TISSUE
	AM- MONIA-N	EASILY HY- DROLYZABLE AMIDE-N	ASPARA- GINE AMIDE-N	AMINO-N (VAN SLYKE)	TOTAL-N	
A	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	
B	0.009	0.22	0.23	1.12	2.82	39.0
C	0.016	0.21	0.19	0.95	1.61	32.1
D	0.353*	0.16*	0.20	0.74	1.46	25.6
						9.8
CRYSTALLINE DEPOSITS 46	NITROGEN PER 100 MGM. OF CRYSTALLINE DEPOSIT†					YIELD IN GM. OF CRYSTAL- LINE DEPOSIT
						<i>gm.</i>
i†	.....	.....	.....	.....	8.1	0.2175
ii†	.....	.....	.....	.....	16.0	0.2575
iii	.....	1.58	6.68	10.5	18.38	2.515
iv	.....	5.11	3.08	12.7	18.53	3.5725
v	0.29	3.74	3.27	10.8	16.9	1.413
vi	0.64	4.61	0.97	11.4	14.9	1.565
vii	0.42	3.70	2.14	11.0	15.85	0.457

\* A loss of easily hydrolyzable amide occurred here due to the fact that after adding the mercuric nitrate reagent there was an interval of 4 hours before the solution was neutralized.

† Crystalline deposit 46.i was recrystallized from water shown to consist mainly of tyrosine; crystalline deposit 46.ii was shown to consist of 53% asparagine and a small amount of tyrosine.

fractions obtained in the treatment of both extracts E.46 and E.45 are shown in table VIII.

#### RECOVERY OF NITROGEN FRACTIONS IN THE VARIOUS EXTRACTS AND DEPOSITS

An inadvertent loss of easily hydrolyzable amide-N from the mercuric nitrate precipitate, in the fractionation of extract 46, reduced the yield so that only 71.5 per cent. of the easily hydrolyzable amide of the fresh tissue was recovered in the crystalline deposits. This was however 98.0 per cent. of that contained in the mercuric nitrate precipitate. In the fractionation of extract E.45 this loss was not incurred, and 93 per cent. (as compared with 73 per cent. in the case of E.46) of the easily hydrolyzable amide-N of

the fresh tissue was contained in the mercuric nitrate precipitate. The yield in the crystalline deposits from E.45 was, however, less quantitative due to the adoption of a less effective method of crystallization, so that the yield of easily hydrolyzable amide-N in the crystalline mixtures fell to 70 per cent. of that in the original fresh tissue. The content of asparagine amide-N and total amide-N in the crystalline deposits also represented about 70 per cent. of the original amount in the fresh tissue. It will now be shown that the easily hydrolyzable amide can be identified as glutamine and that the

TABLE VIII

PERCENTAGE RECOVERY OF THE VARIOUS NITROGEN FRACTIONS DURING ISOLATION OF AMIDES IN LARGE-SCALE EXPERIMENTS

NITROGEN FRACTION	RECOVERY OF NITROGEN FRACTIONS: NITROGEN FRACTION IN THE EXTRACT OR DEPOSITS AS PERCENTAGE OF THAT FRACTION IN THE ORIGINAL AQUEOUS EXTRACT OF THE TUBERS						
	EXTRACT CLARIFIED BY TREATMENT WITH EXCESS LEAD ACETATE (C)		EXTRACT PREPARED BY DECOMPOSITION OF MERCURIC NITRATE PRECIPITATE (D)		CRYSTALLINE DEPOSITS		PURE SUBSTANCES
	E.45	E.46	E.45	E.46	E.45	E.46	E.46
	%	%	%	%	%	%	%
Easily hydrolyzable amide-N .....	97.0	95.5	93.0	73.0	65.0	71.5	66.1 (as glutamine)
Asparagine amide-N ..	86.5	83.0	84.0	87.0	71.5	68.3	60.9 (as asparagine)
Total amide-N .....	89.0	88.0	89.0	82.0	68.5	71.6	62.0
Amino-N (Van Slyke)	91.5	85.0	65.0	66.0	.....	.....	36.3 (as amides and tyrosine)
Total soluble-N extracted (excluding heat coagulum) .....	82.0	79.0	80.0	71.0	37.3	36.6	.....
Total-N .....	62.0	57.0	60.5	52.0	28.2	26.8	.....

yield of pure glutamine obtained from these crystalline deposits represented 66 per cent. of that in the original tissue. The yield of pure asparagine accounted for 61 per cent. of that in the fresh tissue. Of the total amino-N (true amino-N plus amide-N which reacts in the Van Slyke reaction) only 36 per cent. is as yet definitely accounted for (table VIII) so that the identity of the remainder of this part of the soluble-N fraction of potato tuber still presents problems.

#### Separation of pure amides and their chemical identification

Tyrosine (46.I) was the first substance to crystallize from the concentrated solution after decomposition of the mercuric nitrate precipitate. Subsequently mixtures (46.II) of tyrosine and asparagine separated. The separation of the tyrosine and the asparagine from these mixtures depends

upon the very slight solubility of tyrosine in cold water. The further crops of crystals from the mother liquor were mixtures rich in amide nitrogen and contained both forms of amide (46 III-VII).

The crystalline mixtures rich in amide nitrogen (46.III-VII) were submitted to further crystallizations in an endeavor to isolate quantitatively the easily hydrolyzable amides. Mixtures rich in asparagine were repeatedly recrystallized in order to recover any easily hydrolyzable amide-N they contained, whereas the fractions rich in easily hydrolyzable amide were submitted to the minimum number of recrystallizations.

The crystalline material from extract 45 was used for the identification of glutamic acid (isolated as the hydrochloride) and of aspartic acid (isolated as copper aspartate) in the products of amide hydrolysis, and also for a study of the conditions under which the amides could be separated by crystallization from aqueous and aqueous-alcoholic mother-liquors using the more ready crystallizing of the stable amides from water and the relative insolubility of the easily hydrolyzable amides in alcohol. By repeated fractional crystallization, pure samples of tyrosine, asparagine, and glutamine were obtained from the crystalline deposits from E.45. As shown in table V, the methods of analysis give a complete account of the nitrogen of these fractions in terms of easily hydrolyzable amide, asparagine amide, and true amino-N. Data so obtained formed the basis of the treatment of the crystalline deposits from extract 46.<sup>4</sup>

The properties on which the separation of asparagine and glutamine depends and the way in which these were applied to isolate pure glutamine as the easily hydrolyzable amide and pure asparagine as the stable amide are indicated below:

(1) Asparagine is less soluble in water than glutamine. Glutamine is soluble 1 in 28 at 18° C.; asparagine 1 in 47 at 20° C. (2). The solubility of both is increased with rise in temperature. Glutamine, however, may suffer decomposition if heated above 60° C. in aqueous solution.

(2) Asparagine and glutamine do not form mixed crystals (70). Asparagine crystallizes readily from aqueous solution in the presence of impurities, whereas glutamine does not (13, 14). As the purity of the solutions increases by repeated fractional crystallization, glutamine crystallizes more readily.

(3) Both amides are sparingly soluble in alcohol. The solubility of asparagine in 50 per cent. alcohol at 9° C. is about 0.08 per cent. The lower solubility of asparagine accounts for the fact that addition of alcohol (up to 50 per cent. concentration) to a saturated solution of glutamine and asparagine will precipitate a mixture containing a higher proportion of asparagine than the mother liquor.

(4) The crystalline mixtures of the amides obtained from potato con-

<sup>4</sup> At this point in the investigation valuable advice on the technique of isolating glutamine from admixture with asparagine was received from DR. G. W. PUCHER of the Connecticut Agricultural Experiment Station.

tained a "soluble impurity" not included in the amide fraction. Mixtures rich in glutamine but containing a trace of asparagine and of this "soluble impurity" could be purified by extraction with the minimum amount of water necessary to dissolve out these impurities.

(5) The purest preparations obtainable by fractional crystallization could be further purified by treatment with Norit (activated charcoal).

The treatment of the mixtures rich in amide-N, based on the above data, was as follows: the crystalline deposit was dissolved in the minimum amount of water at 60° C. and then set aside in the refrigerator at 8–10° C. Asparagine, or a mixture very rich in asparagine, crystallized out. The filtrate was then treated with not more than an equal volume of alcohol (the amount used being adjusted according to the nature of the crystalline preparation used) and set aside in the refrigerator. Asparagine or a mixture of asparagine and glutamine crystallized out. The filtrate was reduced to a small volume in vacuo at low temperature and then treated with an equal volume of alcohol, chilled, and then treated with a second volume of alcohol. The mother liquor, on storage overnight in the refrigerator at 5° C., gave either crystalline glutamine or a mixture rich in glutamine.

This standard procedure was applied to all of the amide-rich mixtures, and the crops of crystals were grouped into four categories: (1) asparagine; (2) mixtures of asparagine and glutamine which often consisted of spherical masses of glutamine adhering to the large transparent crystals of asparagine (figs. 1, 2); (3) glutamine containing a detectable trace of asparagine; and (4) glutamine free from asparagine. Category 2 was gradually eliminated by repeated applications of the standard procedure. As the crystalline mixtures became purer no sharp separation of glutamine from asparagine was possible, as the glutamine showed an increasing tendency to crystallize out readily. Glutamine-rich fractions were therefore obtained which contained a trace of asparagine and an impurity very soluble in water. To remove both of these impurities the glutamine-rich fractions were treated with a small amount of water. The undissolved residues were crude glutamine. The filtrates were submitted to fractional crystallization until glutamine-rich fractions were again obtained. The identity of tyrosine, asparagine, and glutamine obtained is established by the following data.

#### TYROSINE

The crystalline mixtures (fig. 1) composed of tyrosine and asparagine (46.I,II) yielded by fractional crystallization 0.288 gm. tyrosine and 0.12 gm. asparagine. The tyrosine had a melting point (with decomposition) of 256–258° C. (uncorr.). The elementary analysis found C, 58.6 per cent.; H, 6.14 per cent.; N, 7.6 per cent. Tyrosine requires C, 59.6 per cent.; H, 6.12 per cent.; N, 7.73 per cent.

#### ASPARAGINE

The crystalline mixtures rich in amide nitrogen yielded 2.66 gm. of asparagine. This substance had a melting point (sealed tube, with decom-



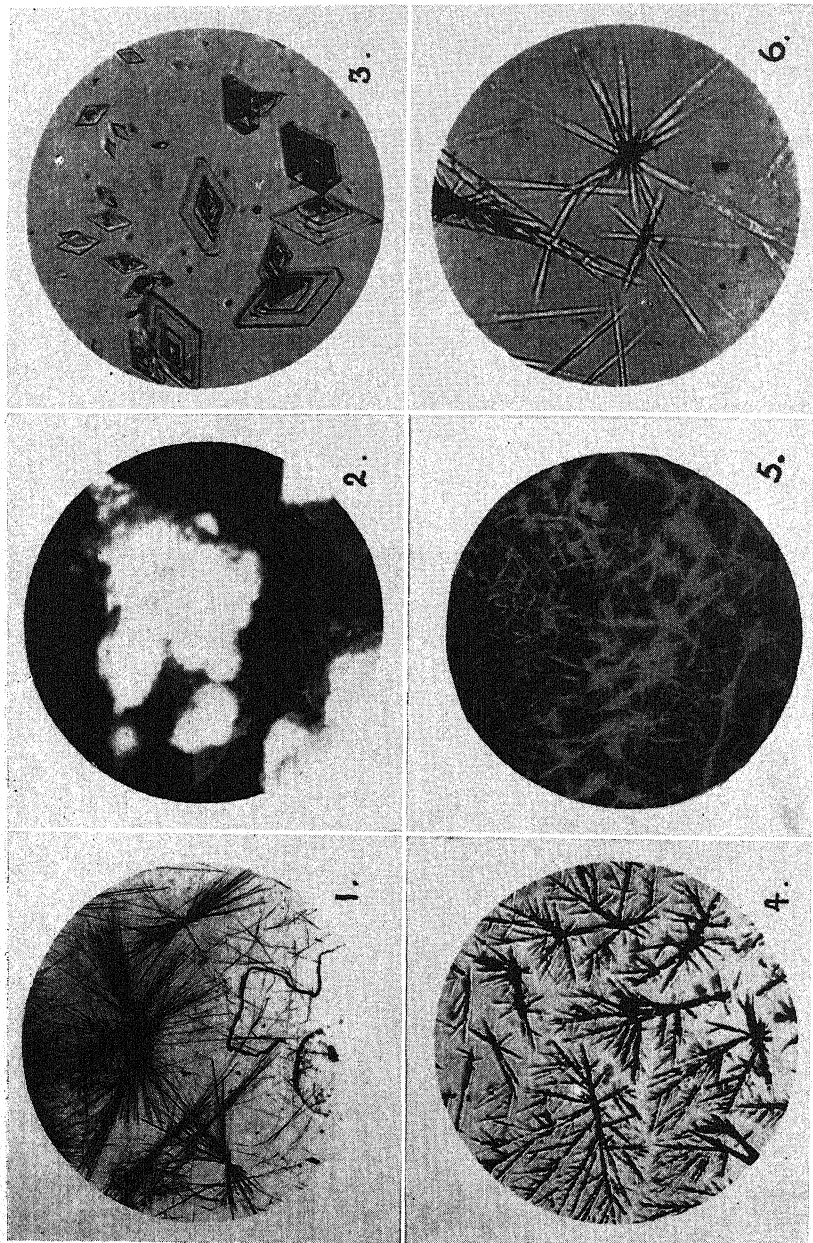


FIG. 1. Tyrosine ( $\times 30$ ). FIG. 2. Mixture of asparagine and glutamine ( $\times 44$ ).  
FIG. 3. Asparagine ( $\times 70$ ). FIG. 4. Crude glutamine ( $\times 48$ ). FIG. 5. Glutamine ( $\times 55$ ).  
FIG. 6. Glutamine ( $\times 175$ ). (Photographs by A. Bates.)

position) of 226° C. (uncorr.). The nitrogen analysis found N, 18.3 per cent.; asparagine-amide-N, 9.1 per cent.; and amino-N (Van Slyke), 9.08 per cent.; the amide hydrolyzed under the conditions of hydrolysis of the easily hydrolyzable amide was only 2.6 per cent. of the total.

This sample of asparagine, dissolved in the minimum amount of water at 70° C. and treated with 100 mgm. Norit for 10 minutes at 70° C. yielded 2.44 gm. of *recrystallized asparagine*. The recrystallized asparagine had a melting point (sealed tube, with decomposition) of 227° C. (uncorr.). The elementary analysis found C, 32.2 per cent.; H, 6.69 per cent.; N, 18.6 per cent.; asparagine-amide-N, 9.3 per cent.; and water of crystallization 12.0 per cent. The identity of pure asparagine is therefore established.

#### GLUTAMINE

The amount of crude glutamine obtained was 3.30 gm. (fig. 4). This had a melting point of 182° C. (uncorr.) and a mixed m.pt. of 181° C. with a sample of pure glutamine at m.pt. of 182° C. The nitrogen analysis found N, 18.7 per cent.; glutamine amide-N, 8.7 per cent.; and amino-N (Van Slyke), 15.2 per cent.; 3.05 gm. of glutamine purified with Norit were obtained (figs. 5, 6). This had a melting point of 184° C. (uncorr.). The elementary analysis of the purified glutamine found C, 39.9 per cent.; H, 6.86 per cent.; N, 19.3 per cent.; amide-N, 9.2 per cent.; and amino-N (Van Slyke), 16.5 per cent. Glutamine requires C, 41.1 per cent.; H, 6.9 per cent.; N, 19.2 per cent.; and amide-N, 9.6 per cent. The optical rotation found was  $[\alpha]^{25}_D = +5^\circ$ . One recrystallization from water yielded 2.71 gm. of recrystallized glutamine with a melting point of 185° C. (uncorr.). The elementary analysis of recrystallized glutamine found C, 40.5 per cent.; H, 6.78 per cent.; N, 19.1 per cent.; amide-N, 9.5 per cent.; and amino-N (Van Slyke), 17.5 per cent. The identity of glutamine as the easily hydrolyzable amide of potato tuber is, therefore, established.

The yields obtained were tyrosine, 0.116; asparagine, 1.42; and glutamine, 1.51 gm. per kgm. of fresh tissue and, as shown in table VIII, the yield of glutamine and asparagine accounts for 66 and 61 per cent., respectively, of the easily hydrolyzable and stable amide-N, present in the fresh tissue.

VAUQUELIN (53) and subsequently HIRSCH and LUDWIG (26) demonstrated the presence of asparagine in the potato. HUNGERBUHLER (28) in 1885 recorded that the mother liquor from a first crystallization of asparagine, when evaporated to dryness, yielded a residue which was not definitely crystalline. This residue, once recrystallized from water, gave on saturating with cupric hydroxide a sky-blue liquid from which a copper compound could be separated. HUNGERBUHLER suggested from the percentage of copper (18.4) that glutamine copper was probably present. NEUBERGER and SAUGER (38) reported, during the course of the present investigation, the isolation, but in very low yield (90 mgm. per liter of expressed sap), of a specimen of glutamine (m.pt. 182° C., N content 18.65 per cent.) from King Edward potato tubers. From an extract of Majestic potato tubers they

were even unable to isolate any glutamine, although their indirect analysis indicated the presence of 1.2 gm. The yield of 1.51 gm. of crystalline glutamine per kgm. of fresh tuber tissue, here recorded, represents an isolation of 66.1 per cent. of that indicated by indirect analysis of the fresh juice and 90.5 per cent. of that contained in the mercuric nitrate precipitate. This recovery of glutamine, in the presence of a greater quantity of asparagine, represents an advance on the best results hitherto recorded (59). The technique of isolation from the mercuric nitrate precipitate has achieved a particularly high yield. An accidental loss of glutamine in the preparation of the mercuric nitrate precipitate obscures somewhat the quantitative yield which is now actually attainable. Having achieved a yield of 93 per cent. of the easily hydrolyzable amide-N in the mercuric nitrate precipitate, as recorded in a parallel extract, it should be possible to obtain 84 per cent. of this fraction as crystalline glutamine.

*The yields thus recorded, when taken in conjunction with the new analytical data presented, indicate that in all probability the whole of the easily hydrolyzable amide fraction of the dormant tubers is glutamine.* There is thus a very strong presumption that when, during active metabolism, the easily hydrolyzable amide content of potato discs increases (49) the increase is due to glutamine. Thus, this more critical account of the analytical determination of this amide fraction, its conditions of hydrolysis, and behavior in the Van Slyke determination, together with its isolation, crystallization, and elementary analysis has vindicated the conclusion, drawn by STEWARD and PRESTON (49) from indirect evidence, that the easily hydrolyzable amide of actively metabolizing potato tissue was probably glutamine.

#### Evidence of the rôle of acid amides in metabolism

Despite much investigation the precise rôle of the acid amides in plants under diverse metabolic conditions is not certain, and the respective rôles of asparagine and glutamine is unknown. SCHULZE and PRIANISCHNIKOW (45) considered that in certain plants glutamine replaced asparagine as the chief amide and fulfilled the same function. VICKERY, PUCHER, and CLARK (62) and CLARK (9) have shown that tomato plants which contained both amides responded to ammonia supply by accumulating glutamine, but that the asparagine did not significantly increase in amount. GREENHILL and CHIBNALL (21a) and CURTIS (12a) have reported the exudation of glutamine from the leaves of grasses receiving nutrients rich in ammonia. MOTHES (36), on the other hand, using bean leaves, recorded asparagine rather than glutamine accumulation in response to ammonium salts. VICKERY, PUCHER, and CLARK (62) have suggested that in any green plant only one of the amides functions for ammonia detoxication and that the function of the second amide is by no means clear.

#### AMIDASES AS REGULATORS OF AMIDE CONTENT

It is tempting to consider that the marked contrast in their stability determines the respective rôles of these amides. Their formation from the

corresponding amino-acids in the plant appears to be governed by amidase or amidases. DERNBY (15) and LEVENE, SIMMS, and PFALTZ (33) concluded that the amides are hydrolyzed by an erepsin. Thus the amide group would be regarded as a peptide group in which one hydrogen atom of the amino group could be regarded as the equivalent of the amino acid residue of a dipeptide. This was endorsed by GROVER and CHIBNALL (22) who investigated the asparagine splitting power of extracts of barley roots and considered it unnecessary to postulate the existence of a specific asparaginase, regarding the action as due to a dipeptidase. GEDDES and HUNTER (19), however, obtained a preparation of yeast asparaginase which split the amide group quantitatively from asparagine, and had a slight activity on glutamine, but was quite inactive to all other acid amides tried. GRASSMANN and MAYR (20) were able to show that yeast asparaginase was an enzyme distinct from yeast dipeptidase and from aminopolypeptidase. There was evidence for a separate glutamine splitting enzyme in fresh autolyzed yeast. According to SCHWAB (48) yeast asparaginase is inactive to glutamine, but both an asparaginase and a glutaminase exist in barley. SCHWAB supposes that the proportion of the two amides in any given plant will be determined by the "active mass" of the enzymes, and he prefers to speak of asparaginase and glutaminase plants rather than of asparagine and glutamine plants.

CHIBNALL (7), using *Lolium perenne*, and VICKERY, PUCHER, and CLARK (62), using beet, have, more recently, shown the existence of active glutaminases in these plants. KREBS (30), working on animal tissues, has produced evidence for the existence of two distinct glutaminases ("brain type" and "liver type"), differentiated on the basis of their inhibition by glutamic acid and by different pH optima. Both enzymes are distinct from asparaginase. The balance of synthesis or decomposition of amide appeared to be influenced by pH and KREBS concluded that, in brain and retina, glutamine synthesis was not reversed since the hydrolysis only occurred in practice outside the physiological range of pH and that glutamine disappeared in these tissues without forming ammonia.

The rôle of amidase in the formation of amides by potato is strengthened by preliminary experiments here recorded which show that potato sap possesses amidase activity which passes to the protein-melanin precipitate formed on dialysis. The potato oxidase which deaminates amino acids also passes to the protein-melanin coagulum, but dialysis robs this preparation both of catechol compounds and of its ability to bring about deamination. In different samples of sap the relative amidase activity towards asparagine and glutamine is not constant, thus indicating that two distinct enzymes may be involved. It remains to be established, therefore, how far the metabolic behavior of the two amides may be interpreted in terms of the respective activity of their corresponding amidases.

#### RÔLE OF OXIDATION: ENERGY REQUIREMENTS IN AMIDE SYNTHESIS

The formation of either asparagine or glutamine from the ammonium

salts of the acid requires an energy supply. Thus the conversion of ammonium aspartate to asparagine at pH 7 involves a standard free energy of synthesis of 3460 cal. ✓ WILLARD (68) even considered that under conditions of adequate nitrogen nutrition the energy from the metabolites available for respiration limited asparagine synthesis in etiolated seedlings. KREBS (30) has shown that in kidney cortex and in brain tissue the synthesis of glutamine is linked with aerobic respiration and that hydrocyanic acid inhibits the synthesis of glutamine to approximately the same degree as it inhibits respiration. Under anaerobic conditions no glutamine synthesis takes place in these tissues.

It is only necessary here to recall that the biochemical survey (49, 50, 51) which prompted this investigation was instrumental in focusing attention upon the frequency with which the salt and oxygen effects upon the respiration of actively metabolizing potato cells found their parallel in effects upon nitrogen metabolism. The tissue first increases its content of glutamine (as we are now entitled to call the easily hydrolyzable amide) as it responds to oxygen supply by increased respiration, and the glutamine tends to reflect every subsequent change in the conditions which affect protein synthesis or respiration. CHIBNALL (7) also refers to observations that oxygen affects amide synthesis in plants. The oxygen which affects amide synthesis may be involved in oxidative deamination of amino-acids and/or in carbohydrate respiration. The necessity of carbohydrate for amide synthesis needs no emphasis, for it may act not only as a source of energy but also provide the carbon skeleton of the amide molecules.

A general concept of the factors which determine amide formation under a variety of metabolic or nutritional states should comprehend a range of circumstances which may extend from: (a) the primary synthesis of amides from an external source of nitrate or ammonia and in cells which, by virtue of their photosynthesis or storage products, have adequate carbohydrate available; to (b) the formation of amide from protein by degradation in senescent cells, which have been depleted of carbohydrate. The work on actively metabolizing potato cells falls into the former category; much classical work on excised leaves and etiolated seedlings as clearly falls into the second.

Under the conditions of protein breakdown it is possible to conceive that the energy for amide synthesis derives from the protein breakdown itself and that accumulation of ammonia in starved detached leaves marks the stage at which the necessary supply of N-free precursors ( $\alpha$ -keto acids) for the carbon skeleton of the amides has been consumed. CHIBNALL (7) has shown that blades of perennial rye-grass infiltrated with the ammonium salt of  $\alpha$ -ketoglutaric acid readily produce glutamine and that the whole of the metabolized ammonia is accounted for as nitrogen of new glutamine, together with a subsidiary amount of new asparagine. Furthermore, it was shown that the disappearance of ketonic acids showed good agreement with the amount of new amide formed and that the amount of  $\alpha$ -ketoglutaric acid



which had disappeared was approximately equal to that demanded by the glutamine synthesized. VICKERY, PUCHER, and CLARK (62) showed that the increase in soluble nitrogen of beet roots treated with ammonium salts was accounted for by the increase in glutamine-N (i.e., by the sum of the amide and amino-N of glutamine) suggesting that the glutamine had been synthesized from N-free precursors.

In actively metabolizing potato cells adequate carbohydrate is available, and its metabolism releases energy far in excess of the requirements of all those concomitant processes (including protein synthesis) known to require energy. Under these conditions N-free precursors of amides may be presumed to arise as intermediate products of glycolysis or by oxidative deamination of stored amino-acids. STEWARD and PRESTON (49, 50, 51) used the tissue in such a way that, usually, it was dependent upon internal sources of nitrogen, and the ammonia for synthesis was shown to arise from amino-acids. However, external sources of inorganic nitrogen also equally well suffice as shown by experiments with nitrates (58) and ammonium salts (unpublished data).

The  $\alpha$ -ketonic acids of potato tissue seem not to have been investigated nor have metabolic experiments involving infiltration of the tissues with either  $\alpha$ -ketoglutaric or oxalacetic acid yet been made. STEWARD and PRESTON (49), however, have shown that the synthesis of protein, the utilization of amino-acids and asparagine, the formation of unstable amides, the utilization of organic acid radicals, and the oxidation phenomena involved in the browning reaction of living cells (which are in turn due to an enzyme system which produces oxidative deamination of amino acids) are all linked with the rate of aerobic respiration and hence with the machinery of salt absorption. A biochemical study of the organic acid metabolism of potato discs, under conditions of rapid salt uptake, such as that being undertaken by HOAGLAND and his co-workers (27) with barley roots, is necessary to establish how far utilization of specific organic acids may be correlated with synthesis and the utilization of the amides.

#### AMIDES DIRECTLY INCORPORATED IN PROTEIN

The main protein present in potato tubers and the only protein fraction to have been extensively studied is the globulin tuberlin. The amino-acid analyses of tuberlin are incomplete. KRIESEL and BELOZERSKY (29) record the presence of 1.05 to 1.02 per cent. amide-N in the ash-free dried samples examined. WINTON and WINTON (71) record the presence of 4.6 per cent. glutamic acid. Aspartic acid has, however, not been identified in the products of hydrolysis, although the amount of glutamic acid present, even if it is all present as glutamine, would only account for some 42 per cent. of the amide-N. The relative requirements for asparagine and glutamine for incorporation as such in the synthesized protein cannot, therefore, be assessed. Potato discs synthesize protein under conditions conducive to active metabolism and salt accumulation (49, 50, 51), but it should not be



presumed that the protein synthesized by these vitally active cells is necessarily identical with the globulin tuberin which is a storage product dissolved in the salts of the cell sap. The relationship between asparagine and glutamine and protein synthesis may be much less direct than their incorporation as such into the molecule of protein so synthesized. It seems rather as though glutamine should be regarded as an active molecule more readily capable than other substances of donating nitrogen to molecules about to be synthesized into protein. The following discussion shows that there is mounting evidence that this may be the case.

#### GLUTAMINE AS AN AMINO-N DONATOR

BRAUNSTEIN and KRITZMAN (3, 4) have demonstrated the presence in muscle tissue of an enzyme system which catalyzes the reversible anaerobic deamination of 1(+) glutamic acid, with the formation of alanine from pyruvic acid thus: 1(+) glutamic acid + pyruvic acid  $\rightleftharpoons$   $\alpha$ -ketoglutaric acid + 1(+) alanine. This enzyme they termed aminophorase. Similarly they showed that aspartic acid could also act as a primary amino donator. Also aminophorase will effect the deamination of monocarboxylic amino acids (e.g., alanine) in the presence of either -ketoglutaric or oxalacetic acids acting as amino acceptors. It appeared that almost any  $\alpha$ -amino-acid could be deaminated to, or formed from, the corresponding  $\alpha$ -keto acid by the aminophorase. Thus amino transfer (transamination or "umaminierung") requires that one member of the reacting pair must be a dicarboxylic keto or dicarboxylic amino-acid. Glutamic acid or  $\alpha$ -ketoglutaric acid are particularly active in this respect (4). KRITZMAN (31, 32) considered that two enzymes are involved: a glutamic aminophorase and an aspartic aminophorase. *The transamination reaction is a mechanism whereby interconversion of amino-acids can take place, and it is of particular interest to our discussion inasmuch as it assigns to the dicarboxylic amino acids a key position in this important biochemical process.*

COHEN (10, 11, 12), however, considers that the transamination reaction is more restricted in its operation than is indicated by the evidence of BRAUNSTEIN and KRITZMAN. The work of VIRTANEN and LANE (67), which ascribes to aspartic acid and to transamination a key position in the nitrogen fixation and metabolism of leguminous plants, has also been criticized by WILSON (69). Nevertheless, the work of SCHOENHEIMER and co-workers (44) on animal tissues and the recent application of the mass isotope technique to the study of protein metabolism in plants (24, 66) supports the theory that the dicarboxylic acids play a central rôle in protein metabolism. It was found that deamination and amination take place continuously. Interchange of amino-N between the free amino-acids and the amino acids combined in the protein must continuously occur. Whenever amino-acids or ammonia artificially enriched in N 15 content are supplied to the organism the content of N 15 in the dicarboxylic acids (particularly glutamic acid) is much higher than in any of the other amino-acids in the tissue, with the

possible exception of the amino-acid in which the N 15 was supplied. If Cohen is correct in regarding the transamination reaction as limited in range, then these results suggest that yet another mechanism must be sought to account for the rapid amino group interchange which is continuously proceeding in the living cell, and in this mechanism glutamic acid must be particularly active. Transaminase systems have not been extensively studied in plants and an examination of potato tissue for aminophorase activity has yet to be undertaken.

#### CANALIZATION OF PROTEIN SYNTHESIS THROUGH GLUTAMINE

Earlier papers on the biochemistry of salt absorption in potato discs (49, 50, 51) have been concerned with the synthesis of protein in the tissue immersed in aerated distilled water and in aerated solutions of potassium and calcium salts. Protein synthesis was greater in potassium salt solutions than in water, and it could be suppressed by relatively strong calcium salt solutions. The nitrogen incorporated into the new protein was accounted for by the loss of nitrogen from the soluble nitrogen fraction. The soluble nitrogen was fractionated into  $\alpha$ -amino-acid-N, heat-stable amide-N (asparagine amide-N), and heat-labile (easily hydrolyzable) amide-N; and the relative utilization of the different fractions as sources of nitrogen for protein synthesis was investigated. In the normal synthesis of protein the nitrogen utilized was mainly derived from the  $\alpha$ -amino acids, and when the nitrogen was derived from this source there obtained the closest parallelism between protein synthesis and respiration. Under conditions of normal protein synthesis the heat-stable amide fraction only contributed a small fraction of the required nitrogen. Although amino-N was utilized in protein synthesis, *the concentration of amino-N did not appear to regulate the rate of synthesis*. The heat-stable asparagine amide-N decreased under the conditions which favored synthesis, but the easily hydrolyzable amide-N was found to increase. When, however, protein synthesis was accentuated, as in the strong potassium solutions, there occurred a marked decrease in the content of easily hydrolyzable amide without any similar effect upon the reserve of stable amides. The easily hydrolyzable amides were therefore tentatively regarded as reactive intermediates between the stable reserves of amino-acids and stable amide on the one hand and proteins on the other. The ammonia for formation of the unstable amides was regarded as having its origin in the oxidative deamination of amino acids, in the hydrolysis of asparagine or in the reduction of nitrate.

The identity of the easily hydrolyzable amide fraction of potato tissue with glutamine, in conjunction with the evidence already discussed, emphasizes the importance of glutamic acid as a donator of amino-N in protein synthesis. Transamination indicates how the amino-N of glutamic acid may be transferred to form other amino acids. As yet, however, we do not know how far glutamine itself accounts for all the glutamic acid which is metabolized since free glutamic acid may be a constituent of the

amino-N fraction which has not yet been fractionated. Whether the amide group of glutamine, which reacts like amino-N in the Van Slyke determination, may also be effective as an amino-N donator in the transamination reaction cannot yet be answered categorically. The only evidence on this point (67a) is inconclusive, but it does indicate greater reactivity of the amide-N of glutamine in this respect, than of asparagine. If the amide, as well as the amino, group of glutamine can enter into the transamination reaction and as, in view of its greater reactivity, the glutamine amide group is also the most labile source of ammonia for amino-acid formation from keto acids, then it becomes evident that this substance is an exceptionally versatile nitrogen donator to nitrogen free compounds. This may well be the key to the special significance of glutamine in protein synthesis and may account for the evident fact that, en route from soluble nitrogen reserves, the course of protein synthesis in the cells of potato tuber is canalized through glutamine.

The present paper has concentrated upon the amide fractions of potato tissue. Further work on the biochemistry of potato tissue under conditions conducive to active metabolism is still required. The need to investigate the amidase activity and the transamination system of potato in relation to its glutamine content arises directly from the present paper. Much, however, still requires to be learned of the identity of the amino-acids and organic acids of the potato tuber and of their relation to protein synthesis and respiration, in order that the biochemistry of potato tissue, under the conditions which are conducive to active metabolism and to the accumulation of salts from dilute solutions, can be adequately comprehended.

### Summary of main conclusions

#### I

For purposes of extracting amides, aqueous extracts are preferable to 70 per cent. alcoholic extracts of fresh tissue. Aqueous extracts are not as selective as alcoholic ones and contain protein coagulable by heat. The amount of the amides extracted is not affected by drying the tissue before extraction. Extracts of dry tissue, however, contain less protein than extracts of fresh. Prior to the determination of the soluble nitrogen fractions protein is removed by heat coagulation. Though the quantity so removed is less than by use of various precipitants, including alcohol, this avoids contaminating the extracts with reagents which require to be removed before the analyses. Alcohol proves to be the most drastic of the protein-N precipitants with the exception of tungstic acid. The nitrogen of the aqueous extracts is not completely accounted for by heat coagulated protein, ammonia-N, easily hydrolyzable amide-N, asparagine amide-N, and true amino-N. It would be consistent with all these facts if the aqueous extracts contained other more complex, probably basic, substances which are absent from alcoholic extracts and disappear on purification. A complete account of the alcohol soluble nitrogen and of the nitrogen content of puri-

fied aqueous extracts can be given in terms of ammonia-N, easily hydrolyzable amide-N, asparagine amide-N, and true amino-N. Methods which give the best results for the determination of these fractions of the soluble nitrogen are described.

Ammonia-N should be determined by distillation under reduced pressure at 40° C.; distillation of ammonia in a micro-Kjeldahl apparatus causes some hydrolysis of amides. The determination of total amide-N presents no difficulty. In determining easily-hydrolyzable, amide-N, however, the hydrolysis of both pure glutamine and the easily-hydrolyzable amides of potato extracts was retarded by alcohol. Therefore, to avoid low results alcohol should be removed by distillation under reduced pressure before determining these amide fractions. It is verified that the true amino-N content of these extracts is obtained by determining amino-N (Van Slyke) and subtracting 80 per cent. of the easily hydrolyzable amide-N. The behavior of the easily hydrolyzable amide-N fraction under these analytical procedures is consistent with this substance being glutamine.

## II

The effect of lead acetate precipitation followed by mercuric nitrate precipitation or precipitation by the Neuberg and Kerb reagent (mercuric acetate) on all the soluble nitrogen fractions was determined as well as the effect of phosphotungstic acid on the nitrogen compounds after liberation from the mercuric precipitates. In the light of these results the procedure for preparing crystalline amides from potato tuber was perfected. It depends upon precipitation of mercuric nitrate compounds from the filtrate after lead acetate precipitation and crystallization from the solutions of the nitrogen compounds prepared by decomposing the mercuric compounds. Crystallization was practiced first from aqueous, then from solutions progressively stronger in alcohol.

Two parallel batches of 2.5 and 3.5 kgm. of fresh tissue were worked up by the method prescribed. At each stage analyses of all the principle nitrogen fractions show the course of the purification; 71.5 per cent. of the easily hydrolyzable amide of the fresh tissue was recovered in the crystalline products. This represented 98 per cent. of this amide in the mercuric nitrate precipitates which in one series contained 93 per cent. of the easily hydrolyzable amide of the fresh tissue; hence it should have been possible, but for one inadvertent circumstance, to obtain crystalline deposits containing 91 per cent. of the easily hydrolyzable amide of the tissue and to have recovered 80 per cent. of the easily hydrolyzable amide-N of the initial extract as crystalline glutamine.

## III

Crystalline products containing the amides were submitted to repeated crystallizations. By a process described, tyrosine, glutamine, and asparagine were prepared in pure form. The yields obtained corresponded to

0.116 gm. tyrosine; 1.42 gm. asparagine; and 1.51 gm. glutamine per 1000 gm. of fresh tuber tissue. This accounts for 66 per cent. of the easily hydrolyzable amide-N contained in the fresh tissue as crystalline glutamine; for 61 per cent. of the stable amide-N of the tissue as crystalline asparagine; and 36 per cent. of the Van Slyke amino-N of aqueous extracts as amide-N and as tyrosine. Elementary analyses, melting points, and analyses of amide- and amino-N content of the crystalline products are all consistent with their identity as the pure substances designated. The yield of glutamine obtained is an advance upon any previous report and the method described should make it possible to isolate glutamine in high yield even when asparagine is present in greater quantity.

#### IV

The identification of the easily hydrolyzable amide fraction of potato as glutamine together with the known reactions of this substance, re-emphasizes the key position already assigned to this amide in protein synthesis.

That synthesis and hydrolysis of the amides may proceed under specific amidases is noted and also that amidase activity may well be influenced by pH (by analogy with enzyme preparations from animal organisms) after dialysis. The amidase activity of potato sap can be distinguished from potato oxidase and its oxidative deamination. In leaves, the quantitative synthesis of glutamine from  $\alpha$ -ketoglutaric acid has been established, but there is little direct evidence for potato tissue beyond the fact that organic acid radicles are consumed during synthesis.

The bearing of glutamine on protein synthesis does not derive from its incorporation, as such, in the protein but is due much more to its ability to donate nitrogen to other molecules about to be synthesized into protein. "Transamination," whereby amino groups are transferred enzymatically from amino-acids of dicarboxylic acids to a keto acid, is important because glutamic acid is particularly active in this respect and may be reconstituted from other amino-acids (e.g., of monocarboxylic acids) which do not otherwise enter into the transamination reaction. It is still inconclusive whether the amide group of glutamine behaves like an amino group under the influence of the enzyme which procures transamination. Be this as it may, the reactive amide group of glutamine is the most labile source of ammonia for synthesis. Therefore, the significance of glutamine seems to be that as the soluble nitrogen passes through this substance the amino-groups become particularly susceptible of being "donated" during amino-acid formation, and the amide group constitutes a particularly labile source of nitrogen for synthesis.

Further investigation of the amidase and the supposed aminophorase systems of potato are necessary, and the nutritional effects of  $\alpha$ -keto acid supply should also be determined. The biochemical survey of actively metabolizing potato discs should now be extended to identify the other components of the amino-N fraction and possibly also the alcohol insoluble

nitrogen compounds. This is necessary to specify the range of substances from which nitrogen is withdrawn and canalized through glutamine en route to the more complex substances.

The work at Birkbeck College was interrupted by war conditions. The junior author was able to complete the experimental work at the University of Manchester where he enjoyed facilities for which thanks are now expressed.

The comprehensive paper by ARCHIBOLD on the chemistry of glutamine and its rôle in animals and plants appeared after this paper was prepared; it will be found in Chemical Reviews **37**: 161-208. 1945.

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# THE EFFECT OF VARIOUS IONS ON GUTTATION OF THE TOMATO<sup>1</sup>

G. J. RALEIGH

## Introduction

In a previous publication (6) attention was called to the fact that in an experiment with tomato plants, those supplied with complete nutrient solution guttated while those in solutions lacking both nitrate and ammonium nitrogen did not guttate. At that time it was thought that the low pH of the solutions might have been responsible for the lack of guttation.

Two additional observations made during the course of later work have suggested that pH alone may not have been responsible. On May 8, 1942, all plants in complete nutrient solutions that had been changed three days previously were guttating markedly while others in solutions that had not been changed were not guttating. In neither instance was the pH low. On May 19, 1942, plants that had been allowed to grow in complete solutions for 18 days but were supplied with additional calcium during the interval were treated as follows by adding concentrated solutions to the depleted solutions:

1. Complete solution (Hoagland solution #2).
2. Complete solution but all nitrogen from urea.
3. Solution lacking nitrogen.
4. Solution lacking phosphorus.
5. Solution lacking potassium.
6. Solution lacking calcium.
7. Check (water only added).

On May 20, a very humid day, guttation water was dropping from all the plants in treatments 1 and 6, and none was visible on any plant in any other treatment. The pH of the urea and of the minus-nitrogen solutions was low (pH 4.6), but that of all of the other solutions was about pH 6 except that of the check solution which was nearly neutral. These observations suggested the possibility that addition of an element to a nutrient solution low in that element might cause guttation of plants that had been checked in growth by the deficiency of the element.

GROSSENBACHER (2) in the course of his work on diurnal fluctuation in root pressure noted that plants transferred to fresh, dilute, but balanced nutrient solutions respired appreciably more and developed much more root pressure than those transferred to dilute  $\text{CaSO}_4$ . HAMNER (3) found that nitrate increased the respiration of tomato and of wheat plants with high carbohydrate reserves. HOAGLAND and BROYER (5) suggested that their finding concerning the effect of carbohydrate reserves on salt absorp-

<sup>1</sup> Paper no. 276. Department of Vegetable Crops, Cornell University, Ithaca, New York.

tion had implications as far as root pressure was concerned. In later work, BROYER and HOAGLAND (1) showed the importance of the relation of root metabolism to the upward movement of salt. Guttation was greater on plants which were originally grown with the smaller supplies of salts and were initially higher in sugars.

### Procedure

With the exception of the experiment with potted plants grown in soil, the strain of tomato used in these experiments was one selected from a single plant in a uniform field of Bonny Best. Seed was grown for two generations on isolated plants. Single plants were grown in crocks of 6-liter capacity in Hoagland's #2 solution with A5 minor elements added<sup>2</sup> until they had reached a height of approximately 130 cm. before they were changed to solutions lacking a single nutrient. They were then allowed to grow in the deficient solution until deficiency symptoms were evident. Care was taken to maintain the supply of all nutrients except the deficient one. This was done by changing the solutions or by the addition of equivalent chemicals. The pH of the solutions lacking nitrogen was adjusted every other day with NaOH. A lack of a test for nitrates in any part of the plant as indicated by diphenylamine was considered evidence of nitrate deficiency. At this stage the iodine test for starch indicated high carbohydrate reserves, as would be expected. The diphenylamine test was not relied on in the single experiment with Hubbard squash in culture solution. In that experiment, yellow color of the lower leaves and markedly retarded growth were used as an index of nitrogen deficiency even though the petioles of the older yellow leaves gave a test for nitrate with diphenylamine.

When deficiency symptoms were noted, the plants were transferred from the greenhouse to an underground room with porous brick walls which had been thoroughly soaked with water prior to the experiment and which were kept wet during the course of the work. Solutions were added, and the time required for guttation was noted. In preliminary experiments it made no difference if a complete solution or a solution of a compound containing the deficient element was added to the deficient solutions. In the work reported here the latter procedure was followed. The relative humidity of the room ranged from 93 to 96 per cent. and the temperature varied within 2 degrees during any one experiment and from 68° to 74° F. for all experiments.

In these experiments it was difficult to find a satisfactory index for amount of guttation. Even though much of the solution guttated by the plants was lost in collecting it by placing a test tube under each drop, that method was considered as satisfactory as any. In all of the following experiments where guttation is indicated as marked, it was possible to collect from 1 to 3 ml. of solution from the plants at one time and to repeat the collection after a few hours. In no experiment, except that mentioned under "THE EFFECT OF NITRATE AND OF AMMONIUM NITROGEN ON GUTTATION" did

<sup>2</sup> The solutions used in these experiments were essentially those given in citation (4).



a plant guttate if grown in a solution deficient in an element and not supplied with that element when the plants were placed in the moist room.

#### EXPERIMENTS WITH NITROGEN

THE EFFECT OF NITRATE AND OF AMMONIUM NITROGEN ON GUTTATION.—Plants grown from seed sown April 9, 1942, were kept in complete solutions until July 25 at which time the solutions were low in nitrogen as indicated by the diphenylamine test. On July 25 they were transferred to dilute solutions lacking nitrogen and on July 29 were placed in the moist room. Solutions of  $\text{NaNO}_3$  and of  $\text{NH}_4\text{Cl}$  were added to the deficient solutions to make the concentration of  $\text{NaNO}_3$  and of  $\text{NH}_4\text{Cl}$  0.006 M in respective treatments. To another lot of 4 plants water was added. As indicated in table I the plants with nitrate added began to guttate after 6 hours. The plants with ammonia added did not guttate as rapidly, but all were guttating in 19 hours. One of the plants allowed to remain deficient in nitrogen showed a very small amount of guttation at 2:30 P.M. but not a sufficient quantity to make it possible to collect any of it, and no more was evident after that

TABLE I

TIME REQUIRED FOR GUTTATION TO BECOME APPARENT AND AMOUNT OF SOLUTION COLLECTED FROM PLANTS FOLLOWING THE ADDITION OF  $\text{NaNO}_3$  AND OF  $\text{NH}_4\text{Cl}$  TO NITROGEN-DEFICIENT SOLUTIONS. EXPERIMENT STARTED 11:00 A.M.

TREATMENT	5:00 P.M.	2:30 A.M.	6:30 A.M.	8:00 A.M.	4:00 P.M.
$\text{NaNO}_3$ —0.006 M	All	.....	.....	.....	.....
$\text{NH}_4\text{Cl}$ —0.006 M 1	.....	X	.....	.....	3.1 ml.
2	.....	.....	X	.....	1.2 "
3	.....	.....	X	.....	0.5 "
4	.....	.....	.....	X	0.1 "

time. None of the other three plants in solutions to which water was added guttated. During the course of the experiment the solutions supplied with  $\text{NH}_4\text{Cl}$  were tested for pH three times and NaOH added to raise the pH to approximately 6.5. The amount of guttation water collected from the plants with  $\text{NH}_4\text{Cl}$  at the 19th hour is shown in table I. The nitrate plants guttated profusely.

Microchemical tests indicated the presence of nitrates and of ammonia, respectively, in the guttated solutions soon after it was possible to collect solutions from plants supplied with nitrates and those in solutions to which ammonium chloride had been added.

The experiment was repeated in May, 1944. The plants were moved to the moist room at 10:00 A.M. At 10:00 P.M. two plants supplied with nitrate were guttating. The plants were not seen again until 8:00 A.M. the following day at which time the other two plants with nitrate were guttating. The plants supplied with  $\text{NH}_4\text{Cl}$  were guttating at 11:00 A.M. and 2:30 P.M. of the second day. The plants in solutions to which water alone was added did not guttate.

On the third day the plants were tested for nitrates, using the xylenol method. The leaflets of the check, the ammonia and the nitrate plants

tested, respectively, 10, 20 and 150 p.p.m. of nitrate nitrogen. Guttated solution collected at that time from the nitrate plants contained 12 p.p.m. The difference in the nitrate content of the check plants as compared with those supplied with  $\text{NH}_4\text{Cl}$  cannot be considered significant due to the inadequate number of tests made.

**EXUDATION FROM DECAPITATED PLANTS SUPPLIED WITH NITRATES.**—In this experiment plants grown from seed sown on May 28, 1942, were kept in complete solutions until July 27 at which time they were transferred to solutions lacking nitrogen. On August 21 nine plants were transferred to the moist room, and the tops of three of the plants were cut off six inches above the root. The other six plants were not decapitated until six hours later when guttation was becoming evident on the three of them in solutions to which  $\text{NaNO}_3$  had been added to supply 0.006 M  $\text{NaNO}_3$ . The total

TABLE II

AMOUNT OF BLEEDING FROM DECAPITATED PLANTS IN NITROGEN-DEFICIENT SOLUTIONS SUPPLIED WITH NITRATE (IN ML.). EXPERIMENT STARTED AUGUST 21 AT 11:00 A.M.

TREATMENT		AUG. 22 8:00 A.M.	AUG. 23 9:00 A.M.	AUG. 24 8:00 A.M.	AUG. 25 8:00 A.M.	AUG. 26 9:00 A.M.	AUG. 28 8:00 A.M.
Nothing added decapitated 5:00 P.M.	1	20	45	81	100	130	154
	2	11	36	64	98	108	116
	3	20	58	84	110	130	161
$\text{NaNO}_3$ —0.006 M decapitated 5:00 P.M.	1	70	150	207	230	270	323
	2	176	392	494	550	600	650
	3	170	360	555	680	890	1215
$\text{NaNO}_3$ —0.006 M decapitated 11:00 A.M.	1	58	154	223	275	335	435
	2	265	427	540	640	720	860
	3	163	318	408	532	642	767

amounts of bleeding for each of the six days following the beginning of the experiment are shown in table II. There was considerable variation in the amount of bleeding within the two treatments receiving nitrate but no significant differences between those that had been decapitated at the beginning of the experiment and those cut off after they began to guttate. No plants with nitrates bled less than twice as much as the maximum amount from a plant in nitrogen-deficient solution. The average of those receiving nitrates was approximately five times greater than those in deficient solutions.

**EFFECT OF NITRATE SOLUTIONS ON THE GUTTATION OF POTTED PLANTS.**—Tomato plants of the Earliana variety seeded February 10, 1943, and set into potting soil on February 27 were selected on the basis of foliage appearance and a lack of nitrate in the foliage (diphenylamine test) and placed in the moist room. At that stage the plants were 27 cm. tall. Eight plants were given 200 ml. of  $\text{NaNO}_3$  (0.012 M) and a similar number the same quantity of distilled water. All of the plants supplied with nitrate were guttating after four hours, and guttation was marked after six hours;

none of those given distilled water guttated during the two days of the experiment.

EFFECT OF NITRATE SOLUTIONS ON GUTTATION OF SQUASH PLANTS.—On July 28, 1944, eight Hubbard squash plants were transferred to the moist room. They were seeded June 20 and grown in nitrogen-deficient solutions from July 20 after having been in complete solutions for twenty days. Sufficient  $\text{NaNO}_3$  was added to the nitrogen-deficient solutions to make the  $\text{NaNO}_3$  content 0.005 M. No nutrients were added to the solutions of the other four plants. Five hours later all of the plants in the nitrate solutions were guttating. The plants in nitrogen-deficient solutions did not guttate during a 2-day period.

#### EXPERIMENTS WITH PHOSPHORUS

On May 4, 1944, eight plants seeded December 20, 1943, were placed in the moist room. They had been grown since March 14 in phosphorus-deficient solutions and were showing phosphorus-deficiency symptoms as evidenced by bronzing of the leaves and reduced growth. Enough  $\text{KH}_2\text{PO}_4$  was added to the phosphorus-deficient solutions of one-half of the plants to make the concentration of  $\text{KH}_2\text{PO}_4$  0.001 M, and the remainder were used as checks. After seven hours one plant given phosphorus was guttating; two more were guttating after twenty hours (not seen in interval); and the fourth after twenty-three and one-half hours. Marked guttation continued after that time. None of the plants in solutions lacking phosphorus guttated.

#### EXPERIMENTS WITH POTASSIUM

Twelve plants seeded April 9, 1942, and grown in complete solutions from May 5 to July 11, were transferred to potassium-deficient solutions on that date. On August 5 they were placed in the moist room. To the solution of 4 of the plants enough KCl was added to make the concentration of KCl 0.006 M, four were supplied with  $\text{KNO}_3$  (0.006 M) and to four, no chemicals were added. None of the plants without potassium guttated. The degree of guttation following the adding of the two salts of potassium is shown in table III.

The experiment was repeated in May, 1944, with similar results except that two of five plants supplied with KCl did not guttate. When these two plants were sectioned longitudinally after the experiment, many brown areas were found in the stems. This necrosis may have been due to excessive potassium deficiency.

#### EXPERIMENTS WITH CALCIUM AND MAGNESIUM

Two experiments with calcium made in 1942 and 1943 and one with magnesium made in 1944 gave negative results. Plants showing marked calcium deficiency or marked magnesium deficiency did not guttate when the respective nutrients were added to the deficient solutions.

## Discussion

The addition of nitrates to nitrogen-deficient solutions caused marked guttation of plants that had grown in such solutions until no nitrate nitrogen was present in any of the tissues as indicated by the diphenylamine test. At that stage, the plants were high in starch as indicated by the iodine test. Presumably, the supplying of nitrate to such plants caused an increase in respiration as shown by HAMNER (3). Ammonium nitrogen, when substituted for nitrate, produced similar results, but the time required for guttation to start was longer. It is difficult to evaluate the part played by the oxygen from the nitrate. The fact that plants in solutions containing nitrates guttated profusely in a shorter time than those in solutions with

TABLE III

TIME REQUIRED FOR GUTTATION TO BECOME APPARENT ON PLANTS FOLLOWING THE ADDITION OF POTASSIUM SALTS TO SOLUTIONS DEFICIENT IN POTASSIUM.  
EXPERIMENT STARTED 10:00 A.M.

TREATMENT	5:30 P.M.	9:15 P.M.	MIDNIGHT	8:00 A.M.
None added	1 ..... 2 ..... 3 ..... 4 .....	..... ..... ..... .....	..... ..... ..... .....	..... ..... ..... .....
KCl—0.006 M	1 ..... 2 ..... 3 ..... 4 .....	Very slight Considerable Slight Considerable	Considerable $\frac{3}{4}$ to marked Slight Marked	Marked Marked Considerable Marked
KNO <sub>3</sub> —0.006 M	1 ..... 2 Slight 3 ..... 4 Slight	Considerable Marked Considerable Marked	Marked Marked $\frac{3}{4}$ to marked Marked	Marked Marked Marked Marked

ammonium nitrogen may have been the result of oxygen supplied by the nitrate. It may be that those given ammonia were delayed by the acidity resulting from its use even though the solutions were made neutral at frequent intervals. Quantities of nitrate and of ammonium nitrogen could be found by microchemical tests in guttated solutions soon after it was possible to collect such solutions. Because transpiration was markedly reduced by the saturated atmosphere in which these experiments were made, it is presumed that the nutrients in the guttated solutions could not have been carried in the "transpiration stream" but were the result of root activity.

The results of the second experiment with K indicate that injuries to some of the plants probably caused by the deficiency of K may have been responsible for a lack of guttation when solutions containing K were added to depleted solutions. Injury may also have been responsible for similar results in the experiments with Ca and Mg, and it is possible that guttation might have occurred had the plants been removed to the experimental room at an earlier stage in the development of deficiency of each of those elements.

No attempt was made to determine the mechanism responsible for guttation. If some specific mechanism is involved, it would seem that it is

activated by any one of a number of elements if its inactivation has been brought about by a lack of that element.

### Summary

Tomato plants were grown in complete solutions and then transferred to solutions lacking nitrogen, phosphorus, potassium, calcium and magnesium, omitted singly until deficiency symptoms were clearly evident. When the lacking nutrient was supplied to the deficient solutions and the plants were kept in a room with relative humidity of about 94 per cent., marked guttation occurred in the plants supplied with nitrogen, phosphorus, and potassium respectively. Guttation did not occur on the plants in the deficient solutions or on those in solutions supplied with calcium or magnesium.

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# LENGTH OF LIFE OF ROOTS OF TEN SPECIES OF PERENNIAL RANGE AND PASTURE GRASSES<sup>1, 2</sup>

J. E. WEAVER AND ELLEN ZINK

(WITH SIX FIGURES)

## Introduction

It is well known that death of the tops of practically all prairie grasses occurs each fall in temperate grasslands where the soil is regularly frozen. Year after year new shoots replace the old ones in this vegetation of long-lived perennials. But as to what portion of the root system is retained and over what period of time, we are almost without information. This maintains despite the fact that much work has been done to increase our knowledge of the root systems of prairie grasses. Since the early studies of WEAVER (6, 7) on their depth and lateral extent, detailed investigations have been made by PAVLYCHENKO (1) upon their rate of growth, total root length, and quantity and quality of root material. The quantity of root material under different grassland climates has been ascertained by SHIVELY and WEAVER (2), and the quantity under different degrees of utilization of these grasses in the same climate by WEAVER and HARMON (8). WEAVER, HOUGEN, and WELDON (9) studied the amount of root material at different soil depths; but the length of life of the roots of prairie grasses, except for brief study by STODDART (4), seems to have been entirely neglected. However, an investigation on the longevity of the seminal roots of certain grasses has recently been made by WEAVER and ZINK (10).

This lack of information is general for other grasses as well. Notable exceptions are the studies of SPRAGUE (3) on Kentucky bluegrass and Colonial bent, and of STUCKEY (5) on twelve species of cultivated grasses. Sprague concluded that at least one-half of the root system is newly generated each spring; Stuckey states that for some species the whole root system is regenerated annually.

## Methods

### CONTAINERS

Plants were grown from seed in large containers. Nine cylindrical cans made of heavy galvanized iron, 24 inches tall and 18 inches in diameter, were filled with loam potting soil. This had been screened, brought to an approximately uniform water content favorable for rapid growth, and thoroughly mixed. The soil had a hygroscopic coefficient of about 10 per cent. Openings in the bottom of the cans, covered with woven wire and an inch

<sup>1</sup> Contribution from the Department of Botany, University of Nebraska, no. 148.

<sup>2</sup> This investigation was aided by a grant to the senior author from The Graduate Council of The University of Nebraska, and grants to the junior author from the Nebraska Academy of Sciences.



depth of gravel, afforded adequate drainage. In filling the containers to near the top, the soil was tamped firmly in place. A removable extension consisting of a heavy galvanized iron band, 6 inches wide, riveted and soldered, and just large enough to fit within the container, was inserted to a depth of 2 inches. Here it rested on a number of rivet-heads just inside the metal rim. This removable extension, which was filled with soil consisting of well-mixed half sand and half loam, increased the depth of the container 4 inches. In addition, 26 heavy galvanized iron containers, 9 by 9 inches in diameter and 29.5 inches deep, were used. Each was fitted with a removable extension, which increased the depth to 33.5 inches, and permitted by its removal and the washing away of the sand-loam mixture easy access to the roots.

Seeds were planted early in March. Those of two species of grasses were planted on opposite sides of each of the large containers and about 4 inches from the side of the extension. Thus, seed of each of 9 species was planted in duplicate but in different large containers. A third planting of each was made in one of the smaller containers. Three groups of each species were required, since the plan was to examine one lot at the end of each of three growing seasons, and it was necessary to destroy the plants in order to examine the individual roots. Also three other smaller containers were planted with seed of the tenth species. In addition, 5 more of the smaller containers were used since it was desired to grow additional plants of certain species. Proper watering and mulching promoted prompt germination and good establishment. Once established, the seedlings were thinned to 5 or fewer in each planting.

#### BANDING

In order to ascertain with certainty the longevity of the roots, a method of banding them individually was devised. Preliminary banding was done in 1929 and methods of procedure outlined. These were successfully used by STODDART (4) in 1932. An opportunity to band roots on a large scale was afforded in the spring of 1943. Bands 8 to 10 millimeters long and 2 to 3 millimeters wide were cut from tin obtained from new, unpainted tubes such as are used for tooth pastes, ointments, etc. The thickness of the sheet of tin was only 0.12 of a millimeter, and hence it was very pliable yet durable. Bands remained bright and unruined even after 3 years in the soil.

The first banding of the roots was done between April 26 and May 14, 1943, when the seedling grasses were 53 to 67 days old. The roots were exposed by removing the extension and washing away the soil by means of a gentle spray of water. It was usually necessary to support the tops of the plants by fastening them to a wire thrust deeply into the soil. Banding was done on damp or rainy days in much subdued light (5 to 10 per cent.), and over a wet floor. Care was taken to keep the roots moist by frequent spraying with a bulb type of hand spray. The grasses were uninjured by this root exposure. At the initial banding all roots 2 inches or more in length (excluding the seminal root or roots) were banded. Usually 2 to 6

roots, varying with the species, were too short to band. The number banded varied from 5 to 74 depending upon the species and the number of seedlings employed. The banding required two persons, one to separate the individual roots one by one from the others. This was done with a hand ice pick. The other person placed a band, which had previously been formed by rolling the tin into an open cylinder around the small end of a pipette, about the root by means of tweezers. It was then gently but tightly rolled between thumb and forefinger until it fit closely around the root (figs. 1, 2). The band partly unrolled if the root grew in diameter. When all the roots were banded, the extension was replaced and the roots covered with a mixture of

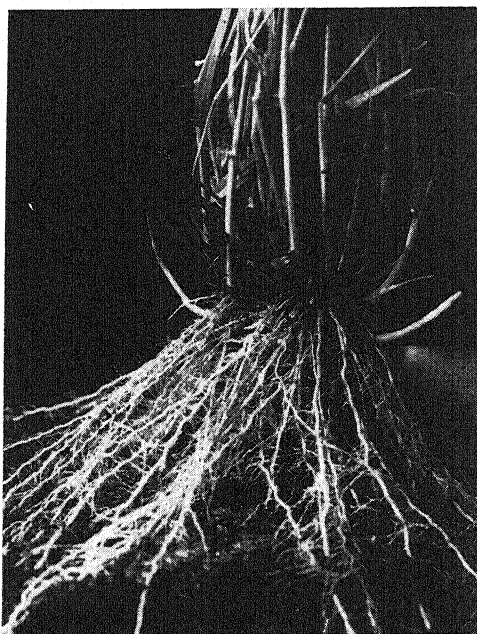


FIG. 1. Roots of switch grass (*Panicum virgatum*) about 3 months old, exposed for banding on June 11, 1943.

dry sand and soil. This readily filled all the spaces about them. It was added gradually and immediately watered by means of a bulb spray. This was repeated until the extension was filled.

A second banding, involving new roots of the same plants that developed later, was made between June 7 and 15, 1943. This was for the purpose of increasing the number of roots under observation. The plants had grown rapidly, tillered abundantly, and some had produced rhizomes. They were 12 to 21 inches tall. A few of the early bloomers had produced flower stalks, and some were flowering. After the extension was removed, a fine, gentle spray of water was used to slowly wash away the soil from the root-mass. Usually all of the new roots were banded, but in some species they were so numerous that only a part of them was used. The total number banded

per species varied between 100 and 419. In plants of most species the new roots formed a peripheral cover which obscured those banded earlier. After banding was completed, a process which required from 30 to 60 minutes or more, dry soil mixed with sand was placed about the roots and immediately watered. Even these larger plants showed no wilting or other signs of injury as a result of the root exposure. It was observed that with several species nothing was gained by banding before the new roots had enlarged considerably and that the same purpose could be accomplished by one banding at a date intermediate to those previously used. Hence, a single

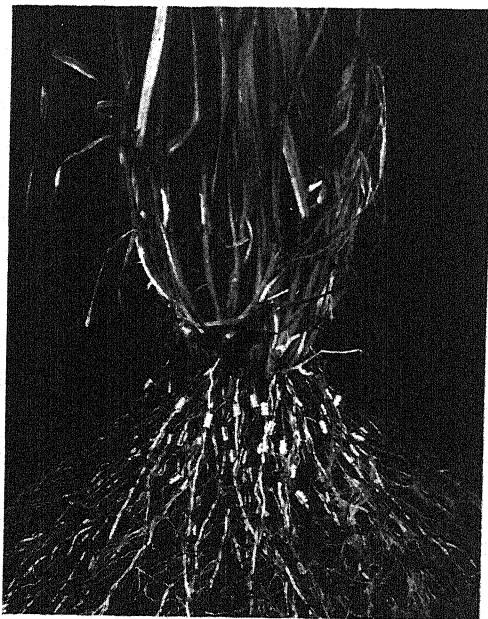


FIG. 2. Roots of crested wheat grass (*Agropyron cristatum*) with numerous bands attached but not yet tightly rolled. It produces an abundance of roots early in life.

banding was made thereafter for plants that were grown to supplement the group employed at the beginning of the experiment.

In 1943, 2,551 roots of 107 plants were banded. The next year roots of several species of grasses were banded where they were growing in the prairie. The following year other lots were banded to ascertain the effect of frequent removal of tops on the longevity of roots.

#### CONDITIONS FOR GROWTH

During the growing season the plants were placed in a greenhouse with panels of glass removed from the sides and with windows and ventilators open. The cement floor was well insulated with a thick layer of dried grass, tubs of water were placed on side benches, and humidity was approximately the same as out-of-doors. The sides of the containers were also well insulated against direct sunlight. Although the light was only 50 to 60 per cent.,

the plants were widely spaced, and all made an excellent growth. All but two species, whose normal time for blossoming is in June, flowered the first season, and all flowered profusely each of the following summers. In autumn, the plants were clipped at a height of 2 inches (as is a mowed prairie), and the containers were placed out-of-doors in a large trench. The bottom of the trench was filled with gravel to furnish good drainage, and moist soil was firmly packed about the containers. The tops, now level with the soil, were lightly mulched with grass as is commonly furnished by the growth following fall mowing in prairie. The soil froze solidly to a depth of several inches each winter and in spring thawed and froze repeatedly. Each spring when growth began the containers were removed to the greenhouse until fall. This was done in order to protect the plants from injury by hail or by grasshoppers or other animals. Here proper care could best be given and especially insurance against waterlogging of the soil during periods of heavy rainfall. Moreover, injury by insects and competition by invading grasses could be more readily detected and prevented. Finally, the roots were much more easily recovered for final examination where each container could be moved about.

#### EXAMINATION OF BANDED ROOTS

In examining the banded roots, the container was placed on a small stout table about 18 inches high, so that it was accessible from all sides. The extension was removed after tapping it repeatedly to free it from the soil. It was sometimes necessary to insert a long-bladed knife between it and the soil. The tops of the plants were then removed at the soil surface. With a gentle stream of water, the mixture of sand and loam was slowly washed away, except for that portion close to the plants. A sharp, long-bladed knife was then used to cut through the soil mass and sever the roots at the level of the top of the container. It was completely loosened from the loam soil below. The top 4 inches of the root system with soil intact about it was then inverted and placed in a white enameled tray 18 by 15 inches in length and width and 2.25 inches deep. The tray was tilted, and the soil below the depth of banding was carefully washed away with a bulb spray or a very gentle stream from a hose.

As the tangled root-ends were exposed, they were removed with a scissors and saved for inspection. Muddy water and loose soil were caught in a tub as they left the tray. Soon the small glistening bands became visible (fig. 3). As soon as one was found the root within it was examined to ascertain whether or not it was still alive. While no completely satisfactory test has been devised to determine whether roots of grasses are living or dead, long experience with the roots of many individuals of each species adds greatly to the accuracy of determination. Living roots often had a yellowish-white or brownish color which was usually very different from the color of the dead ones. They had good tensile strength and were not brittle. The dead roots were easily broken, and tensile strength was low. They were often

smaller, and sometimes they had decayed, and the band lay free in the mass of roots. Once familiar with the roots of these grasses, dead roots could be distinguished with certainty where, as here, several inches of the roots were exposed.

After all the bands were removed from the more distal parts of the roots, two or more inches of the root-ends were again clipped. These were retained with the other clippings. Gentle spraying of the inverted roots revealed the bands near their proximal ends. They too were removed one by one. It was often necessary in older roots to carefully pry the crown apart to regain the last few bands. A record was kept of the total number of bands,

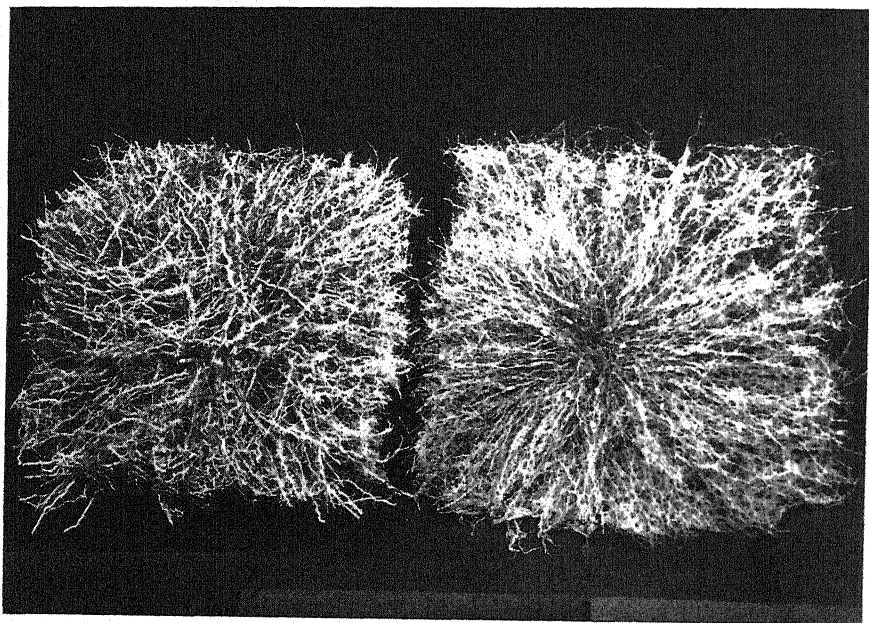


FIG. 3. (Left) Inverted mass of roots of 3 plants of *Panicum virgatum* that were cut from the top of a container after the extension had been removed. The soil has been partly washed from the roots, but they have not been cut back far enough to reveal the bands. (Right) Roots of *Andropogon scoparius*. The finer and more flexible roots of the 3 plants of this species have been pressed back and reveal some of the numerous bands. Both grasses were examined at the end of the second growing season. Each mass of roots is about 9 x 9 inches in diameter.

the number from living roots, and the number from dead ones as well as unattached bands. Since all of the washing was done in the white enameled tray and all soil was plainly visible in a very thin layer, it was only rarely that a band was washed out of the tray. Wash water and all soil were retained, however, until the total number of bands was accounted for.

### Results

#### RESULTS OF FIRST GROWING SEASON AND FIRST YEAR

The number of banded roots of each species and the percentage that died either by the end of the first growing season or by the end of the first year



after banding are shown in table I. Each entry represents a separate group of plants, and where there is only one banding it indicates that new lots of plants were grown during a later season in order to increase the total number of banded roots.

Of the 7 species examined at the end of the first summer, only three showed root losses. These were *Bromus inermis* with 25 per cent. loss in one lot only. Losses in *Agropyron cristatum* were very low, 2 and 5 per cent., but *Stipa spartea* lost 18 per cent. Of roots examined at the end of the first year those of *Bouteloua curtipendula* alone suffered no fatalities; losses in four other species ranged from 16 to 36 per cent. (table I).

TABLE I  
RESULTS OF FIRST GROWING SEASON AND FIRST YEAR

SPECIES*	NUMBER OF ROOTS BANDED		PERCENTAGE OF DEAD BANDED ROOTS	
	FIRST BANDING	SECOND BANDING	FALL, 1943	SPRING, 1944
<i>Andropogon furcatus</i> Muhl. (Big bluestem)	5	13	0	.....
	.....	60	0	.....
<i>Elymus canadensis</i> L. (Nodding wild rye)	26	43	.....	23
<i>Panicum virgatum</i> L. (Switch grass)	23	77	0	.....
<i>Agropyron smithii</i> Rydb. (Western wheat grass)	22	78	.....	36
<i>Andropogon scoparius</i> Michx. (Little bluestem)	11	45	0	.....
	.....	55	0	.....
<i>Bouteloua curtipendula</i> (Michx.) Torr. (Side-oats grama)	29	78	.....	0
<i>Stipa spartea</i> Trin. (Needle grass)	.....	33	18	.....
<i>Bouteloua gracilis</i> (H.B.K.) Lag. (Blue grama)	37	68	0	.....
<i>Agropyron cristatum</i> (L.) Beauv. (Crested wheat grass)	.....	56	2	.....
	.....	60	5	.....
	19	56	.....	25
<i>Bromus inermis</i> Leyss. (Hungarian brome grass)	33	69	25	.....
	.....	80	0	.....
	.....	60	0	.....
	34	66	.....	16

\* The first three are tall grasses, the next four are mid grasses, blue grama is a short grass, and the last two are important cultivated species.

The excellent development of both roots and tops of grasses is shown in figure 4. *Bromus inermis* and *Andropogon scoparius*, both of which produced abundant flower stalks, had developed root systems nearly 3 feet long. There were 57 and 93 roots per plant, respectively. Thus, the mass of roots of the several seedlings in the clump was very large. This reflected the good conditions for growth.

#### RESULTS OF SECOND GROWING SEASON AND SECOND YEAR

The number of banded roots and the percentage of those that died by



fall, or by the following spring of the second year, are shown in table II. The plants were reduced to 9 species, since one lot of *Agropyron cristatum* grew very poorly. By the end of the second summer banded roots of *Andro-*

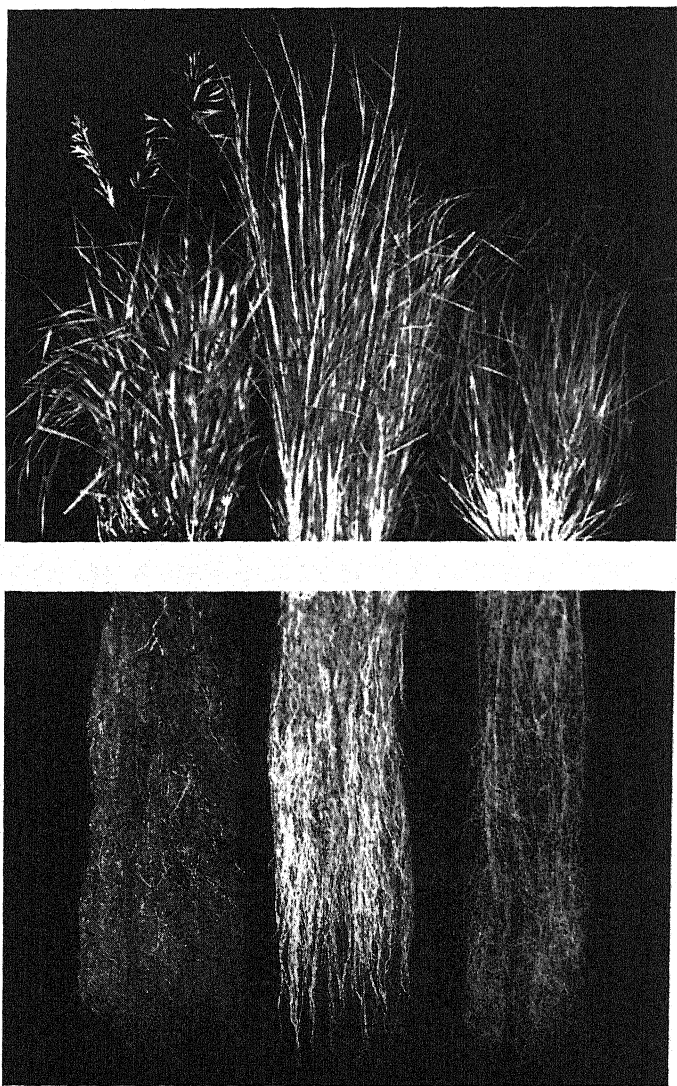


FIG. 4. Tops and deeper roots of one season's growth of *Bromus inermis* (left), *Andropogon scoparius*, and *Stipa spartea* (right). The top 4 inches of the root systems was destroyed in recovering the bands. Depth of root penetration was 32 inches.

*pogon furcatus* and *Panicum virgatum* had suffered no loss and those of *Andropogon scoparius* only 5 per cent. loss. But half or more than half of the banded roots of four other species died. The following spring loss in *Andropogon scoparius* was 31 per cent., which was nearly the same as

TABLE II  
RESULTS OF SECOND GROWING SEASON AND SECOND YEAR

SPECIES	NUMBER OF ROOTS BANDED		PERCENTAGE OF DEAD BANDED ROOTS	
	FIRST BANDING	SECOND BANDING	FALL, 1944	SPRING, 1945
<i>Andropogon furcatus</i> .....	13	10	0	.....
<i>Elymus canadensis</i> .....	35	67	.....	73
<i>Panicum virgatum</i> .....	20	31	0	.....
<i>Agropyron smithii</i> .....	20	47	55	.....
	26	59	60	.....
<i>Andropogon scoparius</i> .....	15	53	5	.....
	31	69	.....	31
<i>Bouteloua curtipendula</i> .....	33	68	.....	64
<i>Stipa spartea</i> .....	17	23	50	.....
<i>Bouteloua gracilis</i> .....	27	89	.....	34
<i>Bromus inermis</i> .....	21	56	64	.....

that of *Bouteloua gracilis*. In *Elymus canadensis* and *Bouteloua curtipendula* the loss was two-thirds or more of the roots banded.

#### RESULTS OF THE THIRD GROWING SEASON

Six species of grasses continued growth during the third summer. Plants of *Panicum virgatum* and *Agropyron cristatum* were accidentally destroyed. Work on *Bromus inermis* was terminated in the fall of 1944, and *Agropyron smithii* spread so widely in the container that it was necessary to remove it in the fall of 1944 in order to save *Stipa spartea* with which it was planted. The losses of banded roots among the six remaining species are shown in table III. They were lowest in *Andropogon furcatus* (19 per cent.), 55 per cent. in *Bouteloua gracilis*, and in four species they varied between 86 and 100 per cent.

#### SUMMARY OF SURVIVAL

The average percentage of survival of each of the 10 species at each period they were examined is shown in table IV. Losses in all species, except

TABLE III  
RESULTS OF THIRD GROWING SEASON

SPECIES	NUMBER OF ROOTS BANDED		PERCENTAGE DEAD BANDED ROOTS
	FIRST BANDING	SECOND BANDING	FALL, 1945
<i>Andropogon furcatus</i> .....	20	60	19
<i>Elymus canadensis</i> .....	17	43	100
<i>Andropogon scoparius</i> .....	43	57	90
<i>Bouteloua curtipendula</i> .....	74	56	86
<i>Stipa spartea</i> .....	12	15	90
<i>Bouteloua gracilis</i> .....	45	45	55

TABLE IV  
PERCENTAGE SURVIVAL AT EACH EXAMINATION

SPECIES	FALL, 1943	SPRING, 1944	FALL, 1944	SPRING, 1945	FALL, 1945
<i>Andropogon furcatus</i> .....	100	.....	100	.....	81
<i>Elymus canadensis</i> .....	.....	77	.....	27	0
<i>Panicum virgatum</i> .....	100	.....	100	.....	.....
<i>Agropyron smithii</i> .....	.....	64	42	.....	.....
<i>Andropogon scoparius</i> .....	100	.....	95	69	10
<i>Bouteloua curtipendula</i> .....	.....	100	.....	36	14
<i>Stipa spartea</i> .....	82	.....	50	.....	10
<i>Bouteloua gracilis</i> .....	100	.....	.....	66	45
<i>Agropyron cristatum</i> .....	97	75	.....	.....	.....
<i>Bromus inermis</i> .....	92	84	36	.....	.....

*Agropyron cristatum*, were gradual. *Agropyron smithii* had a survival at the end of the second summer of 42 per cent., and *Bromus inermis* of 36, but all banded roots were alive on *Panicum virgatum*. Percentage survival at the end of the third growing season was: *Andropogon furcatus* 81, *Bouteloua gracilis* 45, *B. curtipendula* 14, *Andropogon scoparius* 10, *Stipa spartea* 10, but on *Elymus canadensis* none survived.

#### NUMBER OF ROOTS PRODUCED

It is common knowledge that each spring new roots are produced, often in abundance, by perennial grasses, at least in temperate climates. It is important to consider the total number of roots in relation to the death of those produced early in life and the significance of the loss of the latter to the general welfare of the plant. Consequently, when the plants were examined a count of the total number of roots was made, and the average number per plant at different ages was thus ascertained (table V). All counts were of living roots; very few dead ones were found. In general, there was a steady increase in numbers with age. The number per plant was quite large and especially so in *Bromus inermis* (841). The very fine roots of *Bouteloua gracilis* likewise became very numerous (567), but the large, coarse ones of

TABLE V  
AVERAGE NUMBER OF ROOTS PER PLANT AT DIFFERENT AGES

SPECIES	FALL, 1943	SPRING, 1944	FALL, 1944	SPRING, 1945	FALL, 1945
<i>Andropogon furcatus</i> .....	40	.....	228	.....	882
<i>Elymus canadensis</i> .....	307	.....	.....	319	438
<i>Panicum virgatum</i> .....	60	.....	269	.....	.....
<i>Agropyron smithii</i> .....	.....	249	.....	.....	.....
<i>Andropogon scoparius</i> .....	69	.....	248	323	177
<i>Bouteloua curtipendula</i> .....	.....	170	.....	423	378
<i>Stipa spartea</i> .....	24	.....	.....	.....	175
<i>Bouteloua gracilis</i> .....	37	.....	.....	167	567
<i>Agropyron cristatum</i> .....	.....	329	.....	.....	.....
<i>Bromus inermis</i> .....	.....	841	794	.....	.....

*Andropogon furcatus* were even more abundant (882). A single clump of 4 seedlings of *Bouteloua gracilis* produced during three seasons of growth a total of 2,268 individual roots (fig. 5).

In the examination of three-year-old bunches of *Bouteloua curtipendula* it was observed that a few dead rhizomes occurred in the center of the clump.

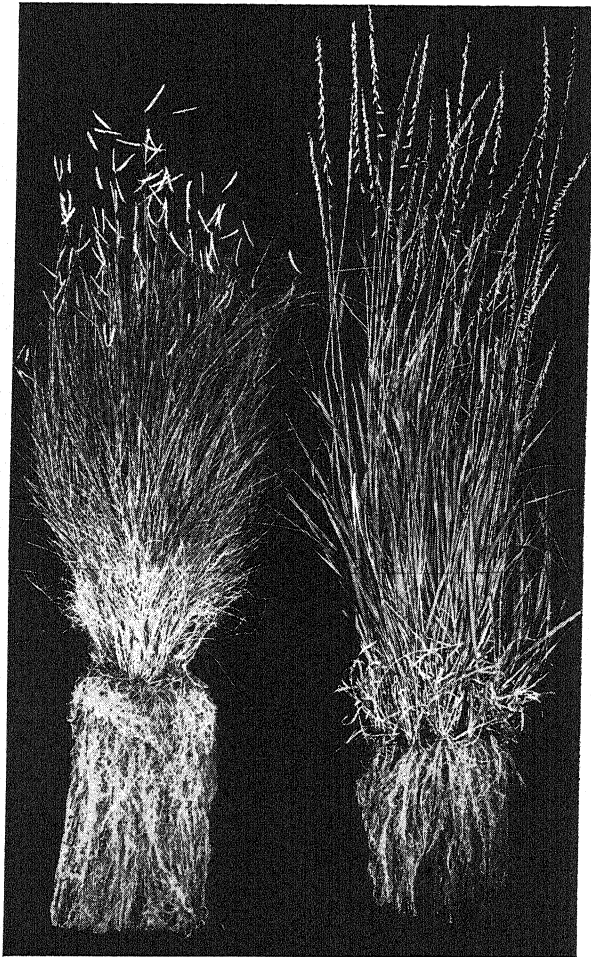


FIG. 5. Bunches of *Bouteloua gracilis* (left) and *B. curtipendula* at the end of the third growing season. In the root system of the first (cut off at a depth of 16 inches) roots occurred at the average rate of 28 per square inch. The four plants in the bunch on the right have a total of 1,512 roots.

This was only 1 to 3 per cent. of the total interwoven lot. Likewise a part of the central and older portion of the crown of *Andropogon scoparius* had died. This, while very small, was the part to which most of the older roots (mostly banded) had been attached. In prairie, degeneration of plants of these species and of many bunch grasses usually begins in the center

of the crown and proceeds outward. Such degeneration would be well advanced in the soil before it would be at all conspicuous above ground, due to the crowding of stem and leaves. This may have been the beginning of the process. Certainly the loss of a few score of roots among hundreds of others would have little effect upon the plant. The writers have determined experimentally that under usual conditions of growth the removal of half or even more of the root system had little harmful effect upon the growth of several species of grasses. Whether or not the roots that develop the second and third years are of longer life-span than those of the first remains to be ascertained.

#### ROOT BANDING IN PRAIRIE

The roots of six species of grasses were banded where they grew in clay loam soil in prairie. This was accomplished with much more difficulty than when the plants were grown in containers. Early in June, 1944, the soil was removed by means of a continuous stream of water from a portable spray pump to a depth of 3 to 4 inches about the roots of four species of bunch-forming grasses. Trenches 18 inches deep were dug in the sod of *Andropogon furcatus* and *Sorghastrum nutans* (L.) Nash. By using the spray of water on a wall of the trench, numerous roots were exposed. A few, as in the bunches, were white and turgid and clearly of the current year's growth. Most of them were one or more years old. The exact age, of course, could not be determined. While the roots were kept moist by frequent sprinkling, the bands were attached to selected uninjured individuals. Moist soil was replaced about them and thoroughly watered. The exact location of the plants was carefully marked by stakes driven into the soil so that the roots could be recovered later by removing a block of sod of minimum size.

In April of the following spring bands were recovered by the washing process already described. This was far more difficult than with sand-loam soil even after the sods were thoroughly soaked in water. Four of the 20 banded roots of *Stipa spartea* were dead, but only 1 among 24 roots of *Adropogon scoparius*. On young bunches of June grass (*Koeleria cristata* (L.) Pers., 6 of the 30 banded roots had died, but 5 among 14 on mature plants.

The remaining bands were recovered from the prairie at the end of the second summer. The number varied from 30 to 60 per species. Losses in one bunch of *Stipa spartea* were 54 per cent., in another 60. Other losses were: *Andropogon scoparius* 23 per cent., *A. furcatus* 45, *Bouteloua curtipendula* 36, and *Sorghastrum nutans* 37. This work was done primarily to ascertain whether or not the method was feasible under field conditions; the losses may or may not be representative of those over a long period of time.

#### EFFECT OF CLIPPING OF TOPS ON LONGEVITY OF ROOTS

The banding method was used in an experiment on the length of life of both seedlings and established plants of several species of grasses. Seeds of



*Bromus inermis* were planted in the large type of container previously described. When the seedlings were 6 inches tall and tillered abundantly (May 10) 130 roots were banded. A week later the plants were clipped at a height of 2 inches (to simulate grazing) and every 10 days thereafter until June 20. After each clipping recovery occurred rather promptly. When the roots were examined on August 2 the plants were 5 to 10 inches tall and in good condition. Only 20 of the banded roots had died, which was 15 per cent. of those originally marked. On the unclipped controls all of the 140 banded roots remained uninjured.

A similar experiment was performed at the same time with *Agropyron cristatum*. It recovered less promptly after clipping and grew poorly. Hence, next to the last clipping was omitted. On August 2 many of the tops below the clipping level were partially dead and dry, but all of the plants had made some new growth, which was 3 to 6 inches tall. Of the 110 roots banded 80 had died. Of the unclipped controls, only 3 per cent. of the 116 banded roots had succumbed. Thus, repeated clipping resulted in a mortality of 73 per cent. of the roots.

Four pieces of sod containing vigorous plants of *Andropogon furcatus*, and 4 with *A. scoparius*, were secured on April 15 from a prairie where the plants were just renewing growth. Each block of sod was 5 inches long, 3 inches wide, and 3.5 inches deep. Each was placed in a separate box on a layer of moist sand and loam 5 inches deep, and soil was compacted about it. The tops grew rapidly and by May 17 abundant new roots 4 to 8 inches long had developed. The soil was now washed from the roots extending below the blocks of sod, the roots banded, and the sods carefully transplanted in the large containers previously used. They contained a mixture of sand and loam which was placed about the roots and sods in a manner that did them no injury.

A total of 53 bands was placed on the new roots of the two lots of plants used as controls and 60 on those of the plants to be clipped. The experimental plants were clipped at a height of 2 inches and the controls at 4 inches immediately after this second transplanting. Clipping, except for the controls, was repeated at intervals of 10 to 14 days until July 8, a total of 5 clippings. The plants recovered promptly after each clipping except the last, *Andropogon furcatus* showing better growth than *A. scoparius*. When examined on August 1, only 3 per cent. of the roots of the controls of *Andropogon furcatus* had died but 45 per cent. of those of the clipped plants. In *A. scoparius* the percentages of death of control and clipped were 3 and 64, respectively. Thus, the injurious effects of the frequent removal of most of the tops is shown by the response of roots as well as by death in the crown, a loss that could be definitely ascertained by the banding method.

#### Discussion

The banding method was found unsatisfactory for some species of grasses with very fine roots. In *Poa pratensis*, *Eragrostis trichodes*, and a few other



species it was difficult to adjust the bands tightly enough to keep other roots from entering the same band. This difficulty was not encountered with most of the native prairie grasses. Also where the number of moderately fine roots was very great and the roots compact, as in *Bromus inermis* and *Agropyron cristatum*, banding was more difficult. That the process of banding or the presence of the bands did not harm the roots is apparent since in most plants all of the banded roots were alive at the end of the first summer and many after a year or more.

Bands were much more readily recovered from bunch grasses than from those producing sods, although certain sod-formers retained the bunch habit for a long time. *Agropyron smithii*, for example, had only a few fine roots

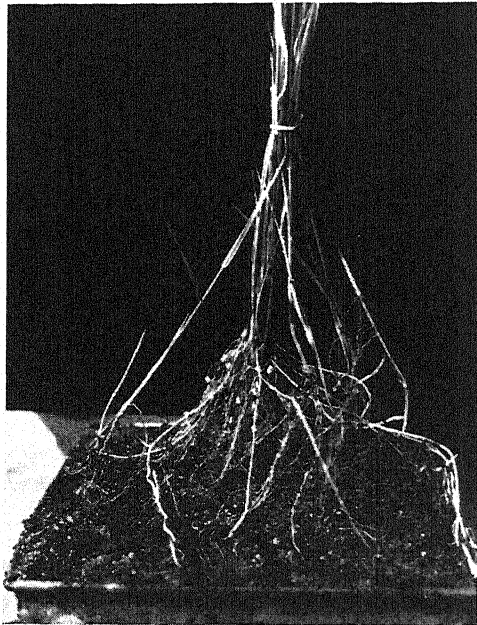


FIG. 6. The first four inches of underground parts of *Agropyron smithii*. The large, widely spreading, white rhizomes are more prominent than the rather small fine roots. This made banding difficult as well as the replacement of the soil without root injury. June 14, 1942.

that supported the seedling plants. Very early several slender rootstocks developed which produced whorls of a few roots each from the nodes, which were an inch or more apart. Hence, the area for banding soon equaled the area of the soil surface (fig. 6). Quite in contrast were the root habits of bunch formers, and even *Andropogon furcatus* and *Panicum virgatum* in early life. They took complete possession of the soil immediately below the crown. The roots of these two species became so large (often two or more millimeters in diameter), even after two or three months, that they were closely compacted at their points of origin from the lower nodes and abundant tillers.

A close check was had on root losses from the two species of *Andropogon* by the minute examination of the roots of about 50 plants of each species each fall. They were grown out-of-doors in large steel drums for experimental purposes. In preparing these washed roots for weighing, the crowns were separated, and from each portion of the crown the roots were cut individually or in such small groups that every root was distinctly visible. They were then finally washed in a white enameled tray where dead or discolored roots could be seen plainly. None was observed at the end of either the first or second growing season. They occurred occasionally the third year.

No losses and replacements of root systems similar to those reported by SPRAGUE (3) and STUCKEY (5) occurred. Sprague examined plants whose roots were almost confined to the first 9 inches of a gray-brown forest soil in New Jersey. He found that one-half of the root system, by weight, was newly generated each year. Stuckey states that for some of the species the whole root system was regenerated annually, with active production of new growth beginning in October. Most of the old roots degenerated shortly after the new ones developed. These species included *Phleum pratense* L., *Festuca elatior* L., *Poa trivialis* L., *Lolium perenne* L., and probably *Agrostis tenuis* Sibth. and *Agrostis alba* L. But on *Poa pratensis* L., *Poa compressa* L., *Agropyron cristatum* (L.) Beauv. and *Dactylis glomerata* L., only a small percentage of roots disintegrated the first year. Although data by STODDART (4) are meager the results with each of the four species of prairie grasses banded are in accord with those of the writers.

Field observations over a long period of years indicate that *Elymus canadensis*, a grass common in moist places, is a species that depends for its permanency of occupation more upon rapid reproduction from seed than upon length of life of the individual. The limited data on *Bromus inermis* and *Agropyron cristatum* indicate considerable permanence of the roots developed the first year. All of the other prairie grasses grown are species of great stability, some individuals probably enduring for a quarter-century or more. While there was a gradual loss of the older roots year by year, the loss is indeed small if not entirely negligible when the great abundance of new roots produced annually is considered. For example, the loss in *Bouteloua gracilis* of 90 per cent. of the roots it produced by June 10 of the first summer amounted after three growing seasons to only 8 per cent. of the total living roots. Similar losses in *Andropogon furcatus* were 2 per cent.

Long life and hence permanency of occupation are characteristics which aid greatly in the adaptation of a grass to semiarid and arid climates. They are likewise important characters of grasses well adapted to soil binding and prevention of erosion.

#### Summary

Seeds of 10 species of perennial range and pasture grasses were planted in triplicate lots in loam soil in containers large enough for ample root

development. A removable extension at the top of each container was filled with sandy loam soil easily washed away when the extension was removed, thus exposing the roots for examination.

Small bands of very thin, pliable sheet tin were placed around individual roots at each of two stages in the development of the plant. A total of 3,424 roots of 181 plants were banded to ascertain their longevity.

The roots were kept moist while exposed and then covered with dry soil which was immediately watered. Some were examined at the end of the first and second year, respectively, and the remainder at the end of each of three growing seasons.

Ninety-seven per cent. of the banded roots of *Agropyron cristatum* survived the first summer and 75 per cent. the first year. Survival on *Bromus inermis* was 92, 84, and 36 per cent. at three examinations. Survival on *Panicum virgatum* and *Agropyron smithii* was, after the second summer, 100 and 42 per cent., respectively. After three growing seasons 81 per cent. of the roots on *Andropogon furcatus* survived, but none on *Elymus canadensis*. Losses in all species were gradual, and after three growing seasons survival of roots was as follows: *Bouteloua gracilis* 45 per cent., *B. curtipendula* 14, and *Andropogon scoparius* and *Stipa spartea* each 10 per cent.

Average number of roots produced by individual plants varied from 175 to 882 at the end of the third summer. Compared with the total number of roots, losses among the banded roots were small to negligible. They often amounted to only 2 to 8 per cent. of the total number of living roots. Nearly all of the species studied have a long life and show great permanency of occupation, both important features in prevention of soil erosion.

Roots of 6 species of grasses of unknown age were banded in clay loam soil in prairie. At the end of a second growing season, losses ranged from 23 to 45 per cent. except in *Stipa spartea*, where they were 57.

Established seedlings of *Bromus inermis* and *Agropyron cristatum* with banded roots were clipped at 2 inches' height at 10-day intervals. There was no loss from unclipped control plants of the first species and only 3 per cent. from the second. But roots of clipped *Bromus* suffered a loss of 15 per cent. and those of *Agropyron* 73.

New roots from vigorous transplanted blocks of sod of *Andropogon furcatus* and *A. scoparius* were banded and the tops of one lot of each clipped at intervals of 10 to 14 days. After 5 clippings losses of roots were 45 and 64 per cent., respectively. The unclipped controls lost only 3 per cent. each.

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# EFFECTS OF POTASSIUM ON THE NITROGENOUS CONSTITUENTS OF *ANANAS COMOSUS*

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(WITH ONE FIGURE)

## General

Results obtained at various times from field experiments have shown that the nitrate content of the tissues of *Ananas comosus* may be increased and the time of floral differentiation delayed by high applications of potassium in the field. These findings, suggesting interrelationships between nitrogen, potassium, plant growth and floral differentiation, are of significance and deserve attention.

This study, concerned with the nitrogenous constituents of the tissues of *A. comosus* (L.) Merr. grown in nutrient solutions containing either high or low amounts of potassium and supplied with equal quantities of nitrogen either as nitrate or ammonium, is intended to furnish information on potassium and nitrogen interrelationships in growth. Ash constituents, chlorophyllose pigments, titratable acidity, and carbohydrates in the same plants were reported in earlier publications (20, 21). The effects of nitrate and ammonium nutrition on the nitrogenous fractions of *A. comosus* were also reported previously (22), but without variation in potassium.

## Review of literature

Potassium-deficient plants may contain in some organs higher amounts of soluble organic nitrogen and lower amounts of protein nitrogen than plants supplied with ample or luxury amounts of potassium. This condition has been variously explained by different investigators. RICHARDS and TEMPLEMAN (13) state that K-deficiency (a) causes rapid disappearance of protein in old but not in new leaves; (b) increases the amounts of amide- and amino-nitrogen; and (c) promotes accumulation of nitrate. WALL (30, 31, 32) claims that K-deficiency curtails protein synthesis at the transitional amino acid to protein stage. BURRELL (1) observed that K-deficiency in soybean plants increased slightly the amounts of amino- and amide-nitrogen but decreased protein nitrogen. NIGHTINGALE *et al.* (8) have shown that in K-deficient tomato plants the amounts of soluble organic nitrogen increased while those of nitrate- and protein-nitrogen decreased. SCHMALFUSS (17) claims that K-deficient plants of *Avena sativa* and *Tradescantia laikenensis* contained higher values of dry matter and soluble organic nitrogen and lower of protein nitrogen than K-supplied plants. The same author also reports that the proportion of soluble- to protein-nitrogen depends on whether

<sup>1</sup> Published with the approval of the Director as Technical Paper no. 162 of the Pineapple Research Institute, University of Hawaii.

the nitrogen in the nutrient solution is supplied as nitrate or ammonium. TURTSCHIN (28) is of the opinion that K-deficient plants are unable to utilize nitrogen as ammonium because of the accumulation of toxic concentrations of  $\text{NH}_4$ -ions and of glucose. HIBBARD and GRIGSBY (7) have found no evidence that increases in potassium or calcium parallels the amounts of nitrogen and protein. HARTT (6) claims that in the blades of 7-month sugar cane plants higher percentages of amino-, protein-, and total-nitrogen were found in potassium-deficient than potassium-supplied cultures, while in the stems the opposite relationship was found to occur. PHILLIPS *et al.* (9, 10, 11) found more solids, reducing sugars, and insoluble nitrogen in K-deficient than in K-supplied plants. ROHDE (14) states that a balance between potassium and nitrogen supply is necessary for efficiency in the assimilation of  $\text{CO}_2$  and use of available light. GREGORY (2) has summarized his views as follows: "Potassium deficiency is characterized by (a) a marked increase in amino- and amide-nitrogen; (b) accumulation of nitrate nitrogen in later leaves; and (c) a very rapid breakdown of protein during senescence of leaves. At emergence the leaves show a normal protein content. It is held that potassium is not primarily associated with protein synthesis but is necessary for maintaining the protoplasmic complex, and in its absence rapid proteolysis occurs. In consequence of this protein breakdown, soluble nitrogen fractions accumulate throughout the plant."

### Methods

The composition of the nutrient solutions, cultural procedures, methods of chemical analyses of the tissues and of statistical treatment of the data have been described in previous publications (19, 20, 22).

The titles of the treatments are reported synoptically in the text. Thus the 205 and 4 mg. of potassium per liter are designated by High-K or high potassium, and Low-K or low potassium, and the nitrate- and ammonium-nitrogen series by N-n and A-n, respectively.

### Observations

#### TOTAL NITROGEN

Total nitrogen, expressed in table II as the sum of the inorganic and organic nitrogen fractions, was higher in the High-K than Low-K cultures, being 1.162 and 1.026 times greater for the N-n and A-n series, respectively. Relative concentration ratios (R.C.R.), obtained by dividing the nitrogen ratio of High-K to Low-K cultures by the respective plant weight ratios, indicate the relative concentrations of various nitrogenous fractions in the High-K cultures with reference to the Low-K cultures. Such ratio values in table II show that the concentrations of total-N in the leaves and stem of the High-K cultures were smaller than those of the Low-K cultures. Similar concentrations in the roots were lower for the High-K cultures in the N-n series but higher in the A-n series.

Inorganic nitrogen, the sum of  $\text{NH}_4 + \text{NO}_3$ , as percentage of total nitro-



gen was 6.10 and 2.50 per cent. in the N-n series and 0.90 and 0.32 per cent. in the A-n series for the High-K and Low-K cultures, respectively. The higher values of inorganic nitrogen in the N-n series resulted from nitrates which are not assimilated until reaching the chlorophyllose tissues of the leaves.

#### TOTAL ORGANIC NITROGEN

This fraction, expressed as the sum of soluble organic-N and protein-N, is reported in table I as mg. per gram of fresh tissue. The data show significant difference between High-K and Low-K cultures in the organic-N content of the leaves (odds 1 : 3000 for the N-n and 1 : 10,000 for the A-n series) and of the stem of the A-n series (odds 22 : 1) but not of the N-n series. Such differences in the leaves in favor of the Low-K cultures were 0.20 and 1.23 mg. per gram for the N-n and A-n series, respectively. The data in table II reporting total amounts of organic nitrogen per plant show that such amounts were higher in the High-K than Low-K cultures because of greater plant weights in the former than the latter. However, it is interesting to note that organic nitrogen values, obtainable from the sum of soluble organic-N and protein-N in table II, differed more between High-K and Low-K cultures in the N-n series (5,543 vs. 4,954 mg.) than in the A-n series (6,289 vs. 6,165 mg.). Such differences may suggest that in the N-n series nitrogen absorption as nitrate was greatly influenced by the concentrations of potassium in the nutrient solution whereas similar influences on ammonium absorption in the A-n series were negligible. Moreover, these findings indicate that  $\text{NH}_4$  and K ions, although of the same electric charge, are not mutually antagonistic in their relationship to absorption by plants.

Relative concentration ratios of total organic-N, obtainable from one-half the sum of soluble organic-N and protein-N values in table II, were smaller for the High-K than Low-K cultures in both series of nitrogen types. Comparison of relative concentration ratio values as obtained above between N-n and A-n series shows that leaf and stem, but not root values, were lower in the latter than former series.

#### SOLUBLE ORGANIC NITROGEN

Soluble organic nitrogen values, reported in table I and figure 1, were higher in the Low-K than High-K cultures. In the leaves the difference of the means of soluble organic-N between High-K and Low-K cultures in favor of the latter cultures, was 0.15 and 0.99 mg. per gram for the N-n and A-n series, respectively. Similar differences in the stem for soluble organic-N, also in favor of the Low-K cultures, were 0.38 and 1.33 mg. per gram for the N-n and A-n series, respectively. Soluble organic-N values per plant, reported in table II, were approximately the same (2,380 vs. 2,294 mg.) in both High-K and Low-K cultures in the N-n series, but in the A-n series those of the Low-K cultures were much greater than of the High-K cultures (3,560 vs. 2,753 mg.). Relative concentration ratio values were generally lower in the High-K than Low-K cultures in both series.

TABLE I

EFFECTS OF 205 VS. 4 MG. PER LITER OF POTASSIUM SUPPLIED TO SOLUTION CULTURES CONTAINING EQUAL QUANTITIES OF EITHER NITRATE-N OR AMMONIUM-N ON THE AMOUNTS (MG. PER GM. OF FRESH TISSUE) OF TOTAL ORGANIC-N, SOLUBLE ORGANIC-N AND PROTEIN-N IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR.

PLANT SECTIONS	NITRATE-N				AMMONIUM-N				
	HIGH-K		LOW-K		HIGH-K		LOW-K		
	TOTAL ORG. N	SOL. ORG. N	PROT. N	TOTAL ORG. N	SOL. ORG. N	PROT. N	TOTAL ORG. N	SOL. ORG. N	PROT. N
Leaves	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Old (B) 1 + 2 (base)	1.21	0.43	0.78	0.97	0.35	0.62	0.96	0.23	0.73
3	1.38	0.56	0.82	1.20	0.37	0.83	1.26	0.29	0.97
4	1.69	0.81	0.88	1.69	0.53	1.16	1.93	0.69	1.24
5 (tip)	1.43	0.66	0.77	1.70	0.46	1.24	2.41	1.02	1.39
Mean	1.43	0.62	0.81	1.39	0.43	0.96	1.64	0.56	1.08
Mature (C) 1 (base)	1.10	0.26	0.84	0.92	0.50	0.42	0.61	0.15	0.46
2	1.07	0.29	0.78	1.06	0.47	0.59	0.63	0.09	0.54
3	1.59	0.65	0.94	1.74	0.79	0.95	1.40	0.52	0.88
4	1.80	0.79	1.01	2.28	1.12	1.16	2.14	0.87	1.27
5 (tip)	2.11	0.82	1.29	2.50	1.06	1.44	2.70	1.34	1.36
Mean	1.53	0.56	0.97	1.70	0.79	0.91	1.50	0.59	0.90
Active (D) 1 (base)	0.65	0.41	0.24	1.12	0.78	0.34	0.46	0.12	0.34
2	0.77	0.53	0.24	0.89	0.43	0.46	0.54	0.16	0.38
3	1.24	0.58	0.66	1.70	0.81	0.89	1.45	0.50	0.95
4	1.85	0.68	1.17	2.20	1.17	1.03	2.22	1.08	1.14
5 (tip)	2.20	0.97	1.23	2.70	1.45	1.25	3.00	1.40	1.60
Mean	1.34	0.63	0.71	1.72	0.93	0.79	1.53	0.65	0.88
Young (E) 1 (base)	1.20	0.59	0.61	1.45	0.71	0.74	1.10	0.71	0.39
2	0.79	0.33	0.46	0.93	0.47	0.46	0.74	0.33	0.41
3	1.23	0.56	0.67	1.41	0.66	0.75	1.28	0.52	0.76
4	1.66	0.72	0.94	2.40	1.16	1.24	2.02	0.86	1.16
Mean	1.22	0.55	0.67	1.55	0.75	0.80	1.28	0.60	0.68
Leaves, mean	1.39	0.59	0.80	1.59	0.74	0.87	1.49	0.60	0.88
Stem									
Base	1.91	0.80	1.11	1.74	0.77	0.97	1.38	0.60	0.78
Middle	2.01	0.80	1.21	2.17	1.28	0.89	1.14	0.60	0.54
Apex	1.92	0.70	1.22	2.35	1.39	0.96	1.46	0.90	0.56
Mean	1.95	0.77	1.18	2.09	1.15	0.94	1.33	0.70	0.63
Roots	1.16	0.20	0.96	1.46	0.32	1.14	1.27	0.35	0.92

TABLE II  
EFFECTS OF "HIGH" VS. "LOW" (205 VS. 4 MG. PER LITER) POTASSIUM ON MEAN PLANT, LEAF, STEM AND ROOT WEIGHTS, AMOUNTS, RATIOS  
AND RELATIVE CONCENTRATION RATIOS (R.C.R.) OF TOTAL-N, SOLUBLE ORGANIC-N, PROTEIN-N, AMMONIUM-N AND NITRATE-N  
OF HIGH-K TO LOW-K CULTURES IN ONE-YEAR-OLD *A. COMOSUS* GROWN IN SOLUTION CULTURES SUPPLIED  
WITH EQUAL AMOUNTS OF NITROGEN EITHER AS NITRATE OR AMMONIUM

ITEMS	UNITS OF MEASURE	NITRATE SERIES				AMMONIUM SERIES			
		High-K	Low-K	RATIO HK/LK	R.C.R. HK vs. LK	High-K	Low-K	RATIO HK/LK	R.C.R. HK vs. LK
Plant weights .....	gm.								
Leaf .....	"	3,855	2,745	1.405	.....	3,895	2,250	1.731	.....
Stem .....	"	3,210	2,280	1.408	.....	3,330	1,820	1.830	.....
Root .....	"	335	173	1.936	.....	342	120	2.850	.....
Total-N .....	"	314	288	1.090	.....	197	310	0.636	.....
Plant .....	mg.								
Leaves .....	"	5,903	5,082	1.162	0.826	6,346	6,184	1.026	0.593
Stem .....	"	5,066	4,221	1.200	0.852	5,631	5,503	1.023	0.559
Roots .....	"	648	407	1.592	0.822	463	347	1.338	0.470
Sol. org.-N .....	"	189	454	0.416	0.382	252	334	0.755	1.186
Protein-N .....	mg.								
Plant .....	"	2,380	2,294	1.037	0.738	2,753	3,560	0.773	0.446
Leaves .....	"	2,063	1,998	1.030	0.732	2,437	3,212	0.759	0.415
Stem .....	"	254	204	1.245	0.643	247	239	1.033	0.362
Roots .....	"	63	92	0.685	0.628	69	109	0.633	0.994
Protein-N .....	mg.								
Plant .....	"	3,163	2,660	1.190	0.847	3,536	2,605	1.357	0.784
Leaves .....	"	2,764	2,139	1.292	0.916	3,146	2,275	1.383	0.756
Stem .....	"	303	191	1.586	0.820	209	107	1.953	0.684
Roots .....	"	96	330	0.291	0.267	181	223	0.813	1.278
Ammonium-N .....	mg.								
Plant .....	"	38	29	1.310	0.932	57	19	3.000	1.735
Leaves .....	"	31	24	1.290	0.916	48	16	3.000	1.640
Stem .....	"	4	2	2.000	1.034	7	1	7.000	2.455
Roots .....	"	3	3	1.000	0.918	2	2	1.000	1.572
Nitrate-N .....	mg.								
Plant .....	"	322	99	3.255	2.320	0	0	0	0
Leaves .....	"	208	60	3.465	2.460	0	0	0	0
Stem .....	"	87	10	8.700	4.500	0	0	0	0
Roots .....	"	27	29	0.930	0.853	0	0	0	0

## PROTEIN NITROGEN

Mean values of leaf protein-N, reported in table I and figure 1, were higher for the Low-K than High-K cultures in both series. The difference of the means between High-K and Low-K cultures, in favor of the latter, was 0.07 and 0.27 mg. per gram of fresh tissue or 8.8 and 30.7 per cent. for the N-n and A-n series, respectively. Similar differences in the stem, but in favor of the High-K cultures, were 0.24 and 0.17 mg. per gram of fresh tissue or 25.5 and 37.0 per cent. for the N-n and A-n series, respectively.

Protein-N values of the leaves, reported in table II and figure 1, in favor of the High-K cultures, were 1.292 and 1.383 times greater for the N-n and A-n series, respectively. Similar values of the stems, also in favor of the

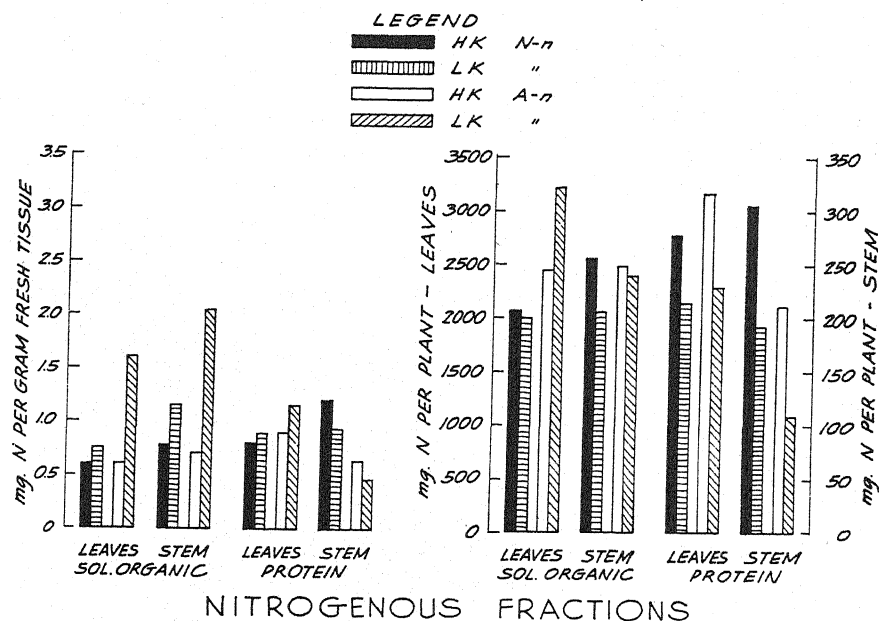


FIG. 1. Total soluble organic-N and protein-N in the leaves and stem of *A. cosmos* grown in solution cultures with 205 p.p.m. of K (H-K) or with 4 p.p.m. (L-K) and either with nitrate (N-n) or ammonium (A-n) nitrogen.

High-K cultures, were 1.586 and 1.953 times greater for the N-n and A-n series, respectively. The data disclose that the rate of conversion of soluble organic-N to protein-N was greater in the stem than in the leaves of the High-K cultures, being 1.228 ( $1.586 \div 1.292$ ) and 1.412 ( $1.953 \div 1.383$ ) times in the N-n and A-n series, respectively. On the basis of information reported elsewhere (22), the higher protein-N and soluble organic-N values in the A-n than N-n series should be attributed to a greater rate of absorption by roots of  $\text{NH}_4$  than  $\text{NO}_3$  ions from the nutrient solution.

Relative concentration ratio values of protein-N, in table II, disclose that in leaves and stem such values were lower in the High-K than Low-K cultures. Similar values of the roots were likewise lower for the High-K than Low-K cultures in the N-n series but higher in the A-n series.

## ALPHA AMINO NITROGEN

Alpha amino-N, a fraction of soluble organic-N determined by the VAN SLYKE (27) gasometric method, reported in table III, was higher in practically all plant sections of the Low-K than High-K cultures in both series. The difference of the means of alpha amino-N between High-K and Low-K cultures, in favor of the latter cultures, was in the leaves 30.2 and 145.6 per cent. and in the stem 21.6 and 82.5 per cent. for the N-n and A-n series, respectively.

Comparison of the above differences of alpha amino-N, in table III, to soluble organic-N, in table I shows that the leaf values were approximately the same for both; i.e., 30.2 vs. 28.3 per cent. in the N-n series and 165.0 vs. 145.7 per cent. in the A-n series for alpha amino-N and soluble organic-N, respectively. However, a similar comparison of stem values shows that they differed more than leaf values; i.e., 21.6 vs. 49.3 per cent. in the N-n series and 82.4 vs. 190.0 per cent. in the A-n series for alpha amino-N and soluble organic-N, respectively. The findings indicate different rates of assimilation or polymerization of alpha amino-N and other fractions of soluble organic-N in leaves and stem.

## INORGANIC NITROGENOUS FRACTIONS

Ammonium nitrogen, reported in tables II and III, ranged in concentrations from 0.005 to 0.019 mg. per gram of fresh tissue in the plants of both N-n and A-n series. Even in the A-n series the ammonium content of roots submerged in nutrient solutions supplied with  $\text{NH}_4$  ions was as low as that of the terminal leaf sections of the same plants and slightly higher than that of similar plant sections in the N-n series. These results, supporting former findings (22), show that  $\text{NH}_4$  ions were assimilated immediately after absorption by the root tissues and changed into compounds of greater complexity. The traces of  $\text{NH}_4$  ions found in all sections of plants supplied either with  $\text{NO}_3$  or  $\text{NH}_4$  ions in solution cultures should, in the opinion of the authors, be attributed to deaminizing processes.

The amounts of nitrate-nitrogen, mostly present in the non-chlorophyllose sections of the leaves, stem, and roots of the N-n series were greater in the High-K than Low-K cultures. Both cultures in the A-n series completely lacked nitrate nitrogen, thus confirming previous findings (22) that nitrification in nutrient solutions is either very little or nil. Nitrate-N was from 1.1 to 4.9 times greater in the basal sections (no. 1) of the leaves of the High-K than Low-K cultures in the N-n series. Similar values of the stem were from 2.5 to 5.7 times higher in the High-K than in the Low-K cultures. Such comparisons between High-K and Low-K cultures indicate that anion absorption ( $\text{NO}_3$ ) was greater from the High-K than Low-K cultures, presumably on account of higher concentrations of cations; i.e., K ions.

## Discussion

Different concentrations of potassium in combination with different sources of inorganic nitrogen in nutrient solutions modified the relative

TABLE III

EFFECTS OF "HIGH" AND "LOW" AMOUNTS OF POTASSIUM (205 VS. 4 MG. PER LITER OF SOLUTION CULTURE) IN NUTRIENT SOLUTIONS SUPPLIED EITHER WITH NITRATE OR AMMONIUM SALTS ON THE AMOUNTS (MG. PER GM. OF FRESH TISSUE) OF ALPHA AMINO-, AMMONIUM- AND NITRATE-NITROGEN IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *Ananas comosus* (L.) MERR.

PLANT SECTIONS	NITRATE-N						AMMONIUM-N					
	HIGH-K			LOW-K			HIGH-K			LOW-K		
	ALPHA-AMINO-N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	ALPHA-AMINO-N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	ALPHA-AMINO-N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	ALPHA-AMINO-N	NH <sub>4</sub> -N	NO <sub>3</sub> -N
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Leaves												
Old (B) 1 + 2 (base)	0.302	0.009	0.294	0.400	0.009	0.060	0.313	0.016	0	0.610	0.007	0
3	0.289	0.007	0.080	0.400	0.009	0	0.291	0.011	0	1.000	0.007	0
4	0.357	0.008	0.018	0.406	0.010	0	0.350	0.013	0	0.730	0.009	0
5 (tip)	0.357	0.010	0	0.392	0.009	0	0.400	0.017	0	1.074	0.013	0
Mean	0.326	0.009	.....	0.400	0.009	.....	0.339	0.014	.....	0.854	0.009	.....
Mature (C) 1 (base)	0.234	0.007	0.285	0.312	0.008	0.133	0.255	0.013	0	0.463	0.013	0
2	0.253	0.009	0.270	0.332	0.009	0.027	0.204	0.012	0	0.708	0.010	0
3	0.297	0.009	0.083	0.447	0.010	0	0.330	0.011	0	0.913	0.008	0
4	0.385	0.009	0.010	0.497	0.010	0	0.378	0.012	0	1.377	0.007	0
5 (tip)	0.344	0.009	0	0.546	0.010	0	0.573	0.013	0	1.636	0.009	0
Mean	0.303	0.009	.....	0.427	0.009	.....	0.348	0.012	.....	1.019	0.009	.....
Active (D) 1 (base)	0.275	0.008	0.285	0.365	0.009	0.157	0.319	0.013	0	0.666	0.011	0
2	0.201	0.009	0.263	0.238	0.009	0.125	0.280	0.011	0	0.494	0.012	0
3	0.297	0.009	0.064	0.365	0.009	0	0.314	0.011	0	0.765	0.009	0
4	0.338	0.009	0	0.447	0.010	0	0.436	0.019	0	0.998	0.007	0
5 (tip)	0.357	0.011	0	0.682	0.014	0	0.560	0.017	0	1.277	0.009	0
Mean	0.294	0.009	.....	0.419	0.010	.....	0.382	0.014	.....	0.840	0.010	.....
Young (E) 1 (base)	0.398	0.012	0.158	0.403	0.016	0.143	0.288	0.018	0	0.627	0.008	0
2	0.302	0.012	0.058	0.282	0.009	0.047	0.302	0.014	0	0.522	0.011	0
3	0.322	0.009	0.016	0.326	0.010	0	0.248	0.012	0	0.472	0.013	0
4 + 5 (tip)	0.352	0.010	0	0.542	0.010	0	0.392	0.012	0	1.000	0.005	0
Mean	0.344	0.011	.....	0.388	0.011	.....	0.307	0.014	.....	0.655	0.009	.....
Leaves, mean	0.315	0.010	.....	0.410	0.010	.....	0.346	0.014	.....	0.850	0.009	.....
Stem												
Base	0.341	0.016	0.135	0.373	0.013	0.054	0.392	0.015	0	0.544	0.007	0
Middle	0.357	0.012	0.193	0.456	0.014	0.054	0.386	0.013	0	0.766	0.011	0
Apex	0.426	0.010	0.385	0.538	0.017	0.068	0.487	0.016	0	1.000	0.017	0
Mean	0.375	0.013	.....	0.456	0.015	.....	0.432	0.015	.....	0.770	0.012	.....
Roots	0.192	0.009	0.086	0.332	0.010	0.100	0.294	0.012	0	0.301	0.005	0



amounts of the various inorganic and organic nitrogenous fractions in *Ananas comosus*. The higher concentrations of potassium increased the nitrate content of the tissues in the N-n series, and, in consequence, the values of total-N, soluble organic-N, and protein-N per plant, reported in table II, were greater in the High-K than in the Low-K cultures. The nitrogen values in table I, reported as milligrams per gram of tissue, are different from those in table II which are presented on a per plant or organ basis. However, the results obtained by either method yield information differentiating the total (table II) from the relative (table I) amounts of the various nitrogenous fractions in the plant.

The rate of nitrogen absorption by roots from nutrient solutions, indicated by the total nitrogen content per plant in table II (sum of ammonium, nitrate, soluble organic-N, and protein-N) for the same growth period, varied in the different cultures. The difference in total nitrogen values between High-K and Low-K cultures in the N-n series was 16.15 per cent. higher for the former cultures; whereas a similar difference in the A-n series, also in favor of the High-K cultures, was 2.6 per cent. The difference in series being considerably smaller in the A-n than N-n series, suggests that the rate of nitrogen absorption by the roots of either the High-K or Low-K cultures was approximately the same or slightly in favor of the former cultures, in spite of the larger root system of the Low-K cultures in the A-n series. But in the N-n series a difference many times greater than in the A-n series indicates that the rate of nitrate absorption by the High-K cultures was higher than by the Low-K cultures. Therefore, the data may indicate that absorption of ammonium nitrogen was not inhibited by either concentration of K ions in the A-n series, but in the N-n series the higher K-ion concentration had increased nitrate absorption.

Protein-N as percentage of total organic-N in the N-n series was 57.1 and 53.7 per cent. for the High-K and Low-K cultures, respectively. Similar values in the A-n series were 56.7 and 42.3 per cent. for the High-K and Low-K cultures, respectively. The difference between High-K and Low-K cultures being smaller in the N-n than A-n series (3.4 vs. 14.4) might indicate in the former series that the rate of nitrate absorption by roots, its assimilation to soluble organic-N, and conversion of the latter to protein-N were coordinated by the supply of K ions. But in the A-n series coordination was lacking between the supply of K ions and ammonium absorption by roots and polymerization of the primary products of assimilation. The apparently regulatory effects of K ions on nitrate absorption by roots retarded the accumulation of much greater amounts of soluble organic-N in Low-K cultures in the N-n series supplied with nitrate, and thus a satisfactory ratio was maintained between soluble organic-N and protein-N. Also, the possible accumulation in plant tissues of  $\text{NO}_3$  ions from external sources but not of  $\text{NH}_4$  ions, except in small amounts, in cultures supplied with nitrate and ammonium, respectively, may influence greatly, by their relative degree of assimilability, the ratio of soluble organic-N to protein-N. There-

fore, the relatively great accumulations of soluble organic-N in both High-K and Low-K cultures in the A-n series should be attributed to the inability of K ions to regulate the absorption of  $\text{NH}_4$  ions by roots. Also, the much greater amounts of soluble organic-N than protein-N in the Low-K than High-K cultures in the A-n series should be attributed to the inadequacy of the low concentrations of K ions to promote conversion of soluble organic-N to protein-N. Doubtless, all reactions involved in the conversion of soluble organic-N to protein-N are of enzymatic nature and may require as substrata for protein synthesis other substances besides the various fractions in the soluble organic-N complex. Therefore, different K-ion concentrations may modify in some obscure manner the rate of enzymatic activity in the conversion of soluble organic-N to protein-N. Such interpretations of the physiological action of potassium are plausible because they are supported in part by the experimental data reported above.

The theories of various investigators on the effects of K ions in protein synthesis are manifold and divergent. STEWART, STOUTE, and PRESTON (25, 26) claim that the stable amide and amino acid fractions in dormant potato tubers were found to be sources of nitrogen for protein formation and that potassium salts appeared to increase and calcium salts to decrease their relative utilization. WALL (29, 30, 31) observed that in K-deficient cultures tomato plants made better growth when supplied with nitrate than with ammonium; the latter, showing rapid breakdown of leaf tissues due to high internal ammonium-ion concentration, increases in amide and amino acid nitrogen and decreases in protein-nitrogen. GREGORY (2), quoting the results of SAÏD (15), SANKARAN (16), and SCHWABE (18) which indicate that the protein content of potassium-deficient leaves was high at first but decreased later, states that protein synthesis as well as hydrolysis must be taking place very rapidly and that the effect of potassium deficiency is related to amino nitrogen content because the level of this fraction determines the rate of proteolysis. This interpretation of GREGORY, although suggesting an additional explanation for the accumulations of amino nitrogen in the Low-K cultures, does not conform in all respects with our experimental evidence, indicating that the protein content of the leaves of the Low-K cultures, in table I, was as high or higher than that of the High-K cultures, suggesting either very little or no proteolysis in the former cultures.

The data in tables I and II reveal that the protein content of the stem was consistently higher in the High-K than in the Low-K cultures. Approximately similar results were obtained by other investigators. WALL's results (29) show that protein nitrogen as percentage of total nitrogen in the upper and lower stem sections of tomato plants grown in cultures with 351, 176, 44 or 0 p.p.m. of K were, respectively, 76.0, 60.0, 69.0 and 51.0 per cent. for the upper stem and 56.0, 55.0, 47.0 and 45.0 per cent. for the lower stem, showing a higher protein content in both stem sections of the plus potassium than minus potassium cultures. BURRELL's (1) findings in soybean plants showed percentage protein values on the fresh weight basis in the stem as 0.233 and

0.167 and in the leaves as 0.125 and 0.147 for the plus-K and minus-K cultures, respectively. HARTT (6) has obtained results indicating that in *Saccharum officinarum* L. protein-nitrogen values of the stem were higher for the plus-K than minus-K cultures and that amino-nitrogen values of the leaves were higher in the minus-K than in the plus-K cultures. The data of NIGHTINGALE *et al.* (8) show in many cases good agreement with the results of this study, but the differences in stem protein-nitrogen between plus-K and minus-K cultures are small. The findings of PHILLIPS, SMITH, and HELPER (11) on the protein-nitrogen content of the stem of tomato plants are partly in disagreement with the data in this study as these investigators claim, "that a moderate but definite degree of potassium deficiency had been reached in the plants not supplied with potassium." Their data show that the K content of the upper-stem tissues of the K-deficient plants was from 50 to 75 per cent. as high as that of the plus-K cultures. In WALL's studies (29) where high and low protein-N in the upper stem of the tomato correlated with the plus-K and minus-K cultures, respectively, the potassium content of the upper stem in the minus-K plants was approximately 34.0 per cent. as high as that of the plus-K cultures. The difference between minus-K and High-K cultures of 50 or 75 per cent., in the study of PHILLIPS *et al.* (11), and 34 per cent., in that of WALL (29), in the potassium content of the tissues, being sufficiently great, might explain the negative results of PHILLIPS.

Unpublished data from experiments with *A. comosus* grown under field conditions and supplied with different amounts of potassium have not fully confirmed the findings reported in this study with respect to the relative amounts of starch or protein in High-K or Low-K cultures. However, the failure of the field-grown plants to yield data comparable to those obtained in nutrient solutions should be attributed to the relatively high initial potassium content of the soil which was many times higher than that supplied to the nutrient solutions of the Low-K cultures. The findings suggest that unless the initial potassium supply in the soil is very low the starch and protein-N content of the tissues of Low-K cultures may be as high or higher than that of High-K cultures on account of restricted growth activity and a lower rate of utilization of the products of photosynthesis and assimilation by the former than the latter cultures.

The above comparisons show that differences in leaf protein-nitrogen between plus-K and minus-K cultures, although in some cases in favor of the former and in others of the latter cultures, fail to correlate in all cases with the amounts of potassium supplied to the roots. However, they show that in the stem and especially in the meristematic tissues of this organ, protein-N was higher in the plus-K than in the minus-K cultures. The meristematic tissues of the peripheral and apical regions of the stem, associated with practically all growth activities in *A. comosus*, contained higher values of protein-N, presumably of protoplasmic origin, in the High-K than Low-K cultures, indicating a positive correlation between high amounts of potassium, protein-N, and plant growth. However, in the highly differenti-

ated tissues of fully expanded leaves a similar correlation was lacking, or a negative correlation could be observed in most sections. STEWART and PRESTON's results (26) with potato discs have indicated that potassium salts stimulate respiration and protein synthesis from stored amino acids while calcium salts depress both processes; also, that amino acids do not yield protein directly but are first deaminated by oxidizing enzymes releasing ammonia and probably a keto acid which contributes to the substrates of aerobic respiration. In a previous study (21), the authors suggested that potassium may also promote the conversion of reducing sugars to sucrose or starch. The details in the physiological function of potassium for promoting the conversion of soluble organic nitrogen fractions to protein nitrogen are not known. However, the manifold activity of potassium suggests that its effects are directly concerned with the activity of the protoplasm which may increase or decrease, presumably by enzymatic action, the rate of sucrose, starch, or protein-N polymerization in certain parenchymatous or meristematic tissues, depending on the physiological status of the plant under the influence of different K-ion,  $\text{NO}_3$ -ion, or  $\text{NH}_4$ -ion concentrations.

Soluble organic-N accumulations in the leaves of the Low-K cultures, attributable by GREGORY (2) to an increased rate of proteolysis, might have resulted from a lower rate of utilization in protoplasm-forming substances on account of much smaller plant weights in the Low-K than High-K cultures. Previous studies (25) have shown that similar accumulations may be found in apparently healthy tissues of plants exposed to adverse conditions. Such accumulations, resulting from translocations of soluble organic-N from injured or dead tissues where it was generated by enzymic hydrolysis of proteins, should not be confused with those in plants lacking symptoms of visual injury or death of tissues. In K-deficient cultures of *A. comosus*, having developed serious injury of the terminal regions of the leaves known as leaf tip necrosis, accumulations of soluble organic-N may result from hydrolysis of the proteins of the dead or injured tissues. However, as differentiation of soluble organic-N fractions, resulting either from hydrolysis of dead tissue proteins or from synthesis by inorganic-N assimilation, is impossible, the accumulations of soluble organic nitrogen in Low-K cultures cannot be assigned definitely to a single source.

### Summary

1. High-potassium cultures (205 mg. per l.) compared with low-potassium cultures (4 mg. per l.), supplied with equal amounts of nitrogen either as nitrate or ammonium, modified the relative amounts of different nitrogenous fractions in the tissues of *A. comosus*.

2. Nitrate nitrogen, as indicated by the nitrate content of the basal leaf and stem sections, was greater in the high-potassium than in the low-potassium cultures in the nitrate series. Ammonium nitrogen, generated possibly by deaminizing processes, occurred in traces in the plants of both nitrate and ammonium series. Ammonium absorbed from nutrient solutions was readily

converted to compounds of greater complexity in the roots, and it is doubtful that any passed from roots to stem unchanged.

3. Soluble organic nitrogen calculated as mg. per gram of fresh weight was higher in the low-potassium than high-potassium cultures of the nitrate series. When calculated as total soluble organic nitrogen per plant, the values were reversed, being greater in the high-potassium than low-potassium cultures. In the ammonium series the values of soluble organic nitrogen, calculated by either method, were greater in low-potassium cultures. Alpha amino-N, a fraction of soluble organic-N, was higher in the low-potassium than in the high-potassium cultures of both series, but in the ammonium series the values were considerably higher than in the nitrate series.

4. Protein nitrogen in the leaves, calculated as mg. per gram of tissue, was greater in the low-potassium than in the high-potassium cultures, but in the stem these values were reversed, being greater in the high-potassium than in the low-potassium cultures. Calculated as total protein nitrogen per organ or plant, the values were greater for the high-potassium than low-potassium cultures in both leaves and stem.

5. The data indicate that the physiological function of potassium in the conversion of soluble organic nitrogen fractions to protein nitrogen in high-potassium cultures is more noticeable in the stem than in the highly differentiated tissues of fully expanded leaves. In the leaves protein-N was higher in the low-potassium than high-potassium cultures. The higher content of protein-N in the stems of the high-potassium than low-potassium cultures is attributed to the conditions in the peripheral and apical regions of the stem which, composed mostly of meristematic tissues and possessing potential growth activity, require greater quantities of proteins for the regeneration of protoplasm than similar tissues of the low-potassium cultures which lack equally great growth potentialities.

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# PHYCOMYCES IN THE ASSAY OF THIAMINE IN AGAR

DOROTHY DAY AND ANNETTE H. HERVEY<sup>1</sup>

## Introduction

In the course of experiments conducted by DAY (1), it was observed that an agar culture with a known quantity of thiamine produced a lesser dry weight of *Phycomyces Blakesleeanus* than was to be expected from a previously determined dry weight of the organism grown in liquid culture with the same quantity of thiamine. The purpose of this investigation was to find out whether or not the use of heat in removing and washing the mycelium grown on the agar media was responsible for this difference in dry weight. The inhibiting effect of agar on the growth of *Phycomyces* also was investigated.

## Materials and methods

*Phycomyces Blakesleeanus* was grown at 25° C. under the conditions used by DAY (1). The basal solution contained per liter of distilled water: 1.5 gm.  $\text{KH}_2\text{PO}_4$ , 0.5 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 50.0 gm. dextrose, 2.0 gm. asparagine, and mineral supplements in p.p.m. as follows: B, 0.005; Cu, 0.02; Fe, 0.10; Mn, 0.01; Mo, 0.01; and Zn, 0.09. Purified Bacto agar and thiamine were added to this basal solution in various proportions and combinations. In each experiment, the mycelium was dried for 24 hours at 100° C. after its removal from the medium by two or more of the following methods with four or eight flasks for each method. 1) Cultures to which hot, distilled water was added were heated until the agar melted. The mycelial mats taken from four similar flasks were washed in two successive beakers of hot (95°—100° C.) distilled water before being placed in an aluminum pan to dry. 2) The flasks were not heated, and cold, distilled water (room temperature) was used throughout. 3) After the addition of cold water to the cultures, the entire contents of four similar flasks were emptied into a Gooch crucible containing a piece of fine cheesecloth as a filter, and one liter of cold water was flooded through the mycelium. 4) A similar crucible with suction was employed, but hot water was used throughout.

## Results

### DRY WEIGHT OF MYCELIUM GROWN IN AGAR MEDIA

The mycelium of *Phycomyces* grown in agar, taken from a flask, and washed in cold water felt slimy before drying; with 1.0 per cent. agar, obvious lumps still adhered to the mycelium. It was impossible to remove all mycelium from the semi-solid agar, as was shown by bits of mycelium floating in the flask when the contents were heated after the mycelium had supposedly been removed. The mycelium filtered and washed with cold

<sup>1</sup> The experimental part of this research was completed at Smith College while both authors were members of the Department of Botany.

water as described in 3 above felt slimy but was less so than that treated by method 2, indicating more successful separation of mycelium from the medium. With hot water, the mycelium did not feel slimy, and the agar appeared to be completely removed by washing in beaker or crucible.

The dry weight of the mycelium grown in agar and washed with cold water was greater than the comparable dry weight of similar mycelium washed with hot water; this relationship was consistent for both pans and crucibles in every experiment regardless of the amount of thiamine added to the culture (table I). The difference was greatest for pan-dried mycelium

TABLE I

DRY WEIGHTS OF MYCELIUM OF *Phycomyces Blakesleeanus* WASHED WITH HOT OR COLD WATER AND DRIED IN PANS OR CRUCIBLES

THIAMINE IN mμ MOLES	WASHED IN COLD WATER			WASHED IN HOT WATER			
	0.1	0.2	0.5	0.1	0.2	0.5	1.0
PERCENTAGE AGAR	MYCELIUM TAKEN FROM FLASK AND WASHED IN PANS*						
0.0 .....	16.1	33.8	70.9	14.5	26.9	55.9	96.4
(liquid)							
0.5 .....	17.0	31.0	78.3	12.6	24.3	58.8	103.2
1.0 .....	32.2	42.0	84.1	12.4	24.5	56.5	110.3
2.0 .....	.....	.....	.....	11.8	21.6	53.0	108.2
3.0 .....	.....	.....	.....	10.7	18.2	49.6	99.9
4.0 .....	.....	.....	.....	10.5	15.1	45.4	94.2
MYCELIUM FILTERED AND DRIED IN CRUCIBLES*							
0.0 .....	18.2	30.7	65.2	15.3	29.4	57.2	.....
(liquid)							
1.0 .....	20.0	38.9	82.7	14.2	26.3	54.3	.....

\* Dry weight is recorded in mg. per culture; each figure represents the average of from 8 to 32 cultures.

from 1.0 per cent. agar, where the mycelium washed in cold water was 49 to 159 per cent. heavier than comparable mycelium washed in hot water (table I). Therefore, it appeared that mycelium grown on agar and washed with cold water had a higher dry weight than did that washed with hot water, apparently because the unremoved agar in addition to the mycelium gave a higher dry weight than the mycelium alone would have given.

#### DRY WEIGHT OF MYCELIUM GROWN IN LIQUID MEDIA

The dry weight of the mycelium washed in cold water was 4 to 27 per cent. greater than similar mycelium washed with hot water (table I). Thus it appeared that washing in hot water lowered the resulting dry weight of the mycelium, probably by dissolving soluble substances out of it. The same effect may be assumed when hot water was used to wash similar mycelium grown in agar.

## EFFECT OF CONCENTRATION OF AGAR ON PHYCOMYCES

Dry weights of mycelium grown with 0.1 m $\mu$  mole and with 0.2 m $\mu$  mole thiamine in two series of cultures containing from 0.0 to 1.0 per cent. agar by 0.2 per cent. steps showed that these amounts of agar had no effect on the growth of the organism. In subsequent experiments with thiamine ranging from 0.1 m $\mu$  mole to 1.0 m $\mu$  mole, the dry weight of mycelium grown in the liquid medium was from 13 per cent. less to 17 per cent. more than the dry weight of mycelium grown with 1.0 per cent. agar (table I). Tests with larger proportions of agar and various amounts of thiamine did not show a perfectly consistent relationship between the amount of agar and the resulting dry weight of the mycelium, but more agar was generally accompanied by less growth of mycelium.

## Discussion

It is clear from these results that the use of cold water in washing *Phycomyces* mycelium which had grown in an agar medium did not result in the complete removal of the agar. Sufficient agar remained to affect materially the dry weight of the mycelium. Although the use of hot water in washing the mycelium grown in the agar medium resulted in the successful removal of the agar, enough soluble material was leached from the hyphae during the washings to influence the dry weight. While these results were to be expected, it was not possible to estimate the amount of the effects without the actual determinations. The differences found are sufficiently great to be significant if *Phycomyces* is used for the quantitative determination of thiamine.

The reduction in growth demonstrated with the higher concentrations of agar may have been caused by the physical resistance of the agar to the growth of the organism, the lessened availability of water, thiamine, or other components of the medium, or the presence of some unknown toxic factor in agar.

## Summary

*Phycomyces Blakesleeanus* was grown in a basal medium of distilled water, minerals, dextrose, and asparagine plus purified agar and thiamine in various proportions and combinations. The mycelium was harvested by the use of hot and cold water in methods which included the use of aluminum pans and Gooch crucibles.

With the basal liquid medium the dry weight of mycelium washed with cold water was higher than that washed with hot water, probably because of the action of hot water on the mycelium in dissolving substances from it.

The higher dry weight of mycelium grown on agar and washed with cold water as contrasted with that washed with hot water might be explained not only by this action of hot water but also by the incomplete separation of the agar and the mycelium with cold water.

Reduced growth of *Phycomyces* was generally correlated with an increased amount of agar.

Grateful appreciation is expressed to W. J. ROBBINS for critical reading of the manuscript.

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## LEAFSPOT OF PEANUT ASSOCIATED WITH MAGNESIUM DEFICIENCY

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W. B. TISDALE

(WITH TWO FIGURES)

It is generally recognized that the susceptibility and prevalence of many plant diseases are associated with plant nutrition. The peanut plant is susceptible to leafspot infection which becomes economically important during the last half of its growth period when the crop is planted in the spring. The severity and rapid spread of infection in the field may not be due to the mere presence of the organism, which is ubiquitous, but to the susceptibility of plants suffering from improper nutrition in certain stages of their growth. Results herein reported of a nutritional study with the Dixie Runner variety of peanut support this assumption.

The experimental peanut plants were grown in sand culture with the roots of each plant in a single gallon glass jug which completely isolated the roots from the fruiting or pegging zone of the plant (fig. 1). The nutrients were applied to the rooting medium by the continuous flow method of SHIVE and STAHL (6), while the nutrients on the pegging zone were applied on the surface and by feeding through the base of the box. For 75 days after germination the rooting and fruiting zones of all cultures were supplied HOAGLAND'S (4) no. 1 complete nutrient solution modified by reducing the concentration of the macronutrients by half and the micronutrients to one-tenth. Ferrous iron was added as needed. At the end of that period the following nutrient treatments were begun on the rooting medium: complete, minus phosphorus, minus potassium, minus calcium, minus magnesium, minus sulphur, and minus micronutrient elements. The complete nutrient solution was applied to the fruiting medium throughout the experiment.

There were three replicates of single plants to each treatment and the roots of each culture received 1.5 liters of nutrient solution daily; the pegging region received 3.5 liters of solution weekly. Plants were grown 21 days in the greenhouse after germination and then because of high temperature were moved outside under a frame covered with one thickness of cheesecloth. This report will deal only with the results obtained with the magnesium-deficient cultures, since these plants were the only ones that developed the leafspot disease to any appreciable extent under the conditions of this experiment.

The leaves of the minus-magnesium plants showed interveinal chlorotic centers with green margins 24 days after magnesium was withheld from the roots. However, the effect appeared initially on the first leaf below the growing tip of the oldest branches. This is in contrast to the typical magnesium deficiency on other plants which usually occurs first on the basal



leaves. At the end of 35 days the deficiency symptom was apparent on the young leaves of all branches including the main shoot and had progressed four to five leaves below the growing tips of the oldest branches. The chlorosis of the affected leaves became more severe with advancing age.

On July 28, when plants were 103 days old, and magnesium had been withheld for 28 days, leafspot was observed on those leaves which first showed symptoms of the nutritional disturbance. A fungus (*Mycosphaerella arachidicola* W. A. Jenk.) was isolated from the spots. The progress of the disease followed the same order of development as that of the deficiency symptom, affecting first the young leaves of the oldest branches and then

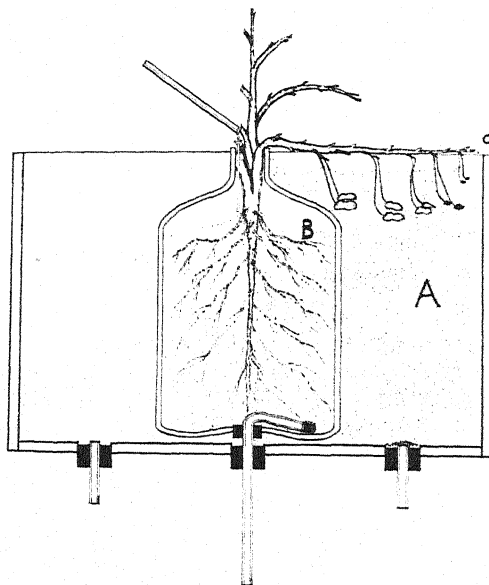


FIG. 1. Diagrammatic view of peanut plant showing: A, fruiting medium; B, rooting medium; and C, string mesh to prevent contact and rooting of branches in the sand.

proceeding to the young growth of all branches. Leafspot on plants in the field usually attacks the basal leaves first and advances progressively toward the tips of branches.

Leafspot counts were made to determine the incidence of disease on the plants of the minus-magnesium and complete-nutrient solutions on August 10, when plants were 116 days old and magnesium had been withheld for 41 days (table I). The counts were made on the terminal and basal leaves of the same branch, and the disease was highly correlated with the area of the deficiency symptom (fig. 2).

Magnesium determination by the LINDNER (5) method was made of basal, central, and terminal leaves from branches of the same physiological age (table II). The leaf material for analysis was collected at the time the leafspots were counted.

The chemical analyses showed a decrease in magnesium concentration

TABLE I

INCIDENCE OF LEAFSPOT ON PEANUT PLANTS GROWN IN COMPLETE NUTRIENT SOLUTION  
AND A SOLUTION WITH MAGNESIUM WITHHELD FOR 41 DAYS  
(AGE OF PLANTS, 116 DAYS)

TREATMENT	No. OF PLANTS	No. OF BRANCHES	No. OF LEAFLETS	NUMBER OF LEAFSPOTS	
				TERMINAL LEAFLETS	BASAL LEAFLETS
Complete nutrient solution .....	3	15	180	6	15
Minus magnesium .....	3	15	204	3502	30

from the basal to the terminal leaves, and spectrographic analyses substantiated those results.

No precaution was taken to prevent spread of the leafspot. Moreover, daily flower counts were made on all cultures when the foliage was wet from dew or rain and close spacing of plants caused branches from other cultures to overlap those of the magnesium-deficient plants. The prevalence of leafspot on plants grown on a deficiency of the elements other than magnesium was no greater than that of the controls even though the given deficiency was severe, and the plants were much weakened.

The plants which had magnesium withheld from the roots had many fruit developing in the fruiting medium which contained magnesium. BURKHART and COLLINS (2) have shown that the growing peanut fruit is capable of absorbing minerals. They further state that gypsum applications condition the peanut plant in such a manner that the foliage becomes very susceptible to leafspot injury.

CHAPMAN (3) states that prolonged magnesium deficiency of citrus

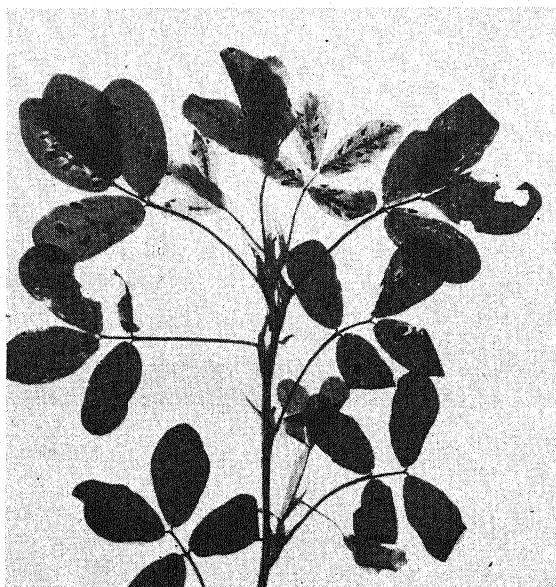


FIG. 2. Branch from peanut plant showing prevalence of leafspot on chlorotic leaflets on August 10, after withholding magnesium from the rooting medium for 41 days.

TABLE II

MAGNESIUM IN PER CENT. OF DRY WEIGHT OF PEANUT LEAVES FROM DIFFERENT POSITIONS ON PLANTS GROWN IN MAGNESIUM-DEFICIENT AND COMPLETE-NUTRIENT SOLUTIONS

POSITION OF LEAVES ON BRANCH	MINUS MAGNESIUM	COMPLETE
	%	%
Terminal .....	0.012	0.307
Central .....	0.022	0.317
Basal .....	0.082	0.401

grown in solution culture brings on typical iron chlorosis in the leaves. BENNETT (1) suggests that the availability of iron might be influenced by other factors. Liberal amounts of ferrous iron were supplied to the rooting and fruiting mediums of the minus magnesium peanut plants in this experiment. Furthermore, there was no response to iron or other micronutrients when solutions were streaked or spread on the surface of the chlorotic leaflets. While the nutrient disturbance was associated with magnesium deficiency, it is entirely possible that it was caused by an interaction of ions.

Visual symptoms of magnesium deficiency of field-grown peanuts have not been observed. However, that does not preclude the possibility that leafspot is induced by a deranged metabolism resulting from magnesium deficiency. In this study it appears that the low level of magnesium was either directly or indirectly responsible for the susceptibility of peanut to leafspot. The exact nature of the nutrient disturbance, as well as whether or not the severity of leafspot can be reduced by higher levels of magnesium in the plant, must await further experimentation.

The authors wish to thank MR. THOMAS C. ERWIN of the Department of Soils for the spectrographic analyses.

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## NOTES

**The St. Louis Meeting.**—The American Society of Plant Physiologists held its twentieth annual meeting at Saint Louis, Missouri, on March 28–30. The Statler Hotel was the official headquarters and the majority of the meetings were held there. Most of the papers were presented in a series of two parallel sessions jointly held with the Physiological Section of the Botanical Society of America. In addition, a joint session was held with Section G, Botanical Society of America, American Phytopathological Society, Mycological Society of America, and the Sullivant Moss Society on Thursday afternoon and a second joint session was held with Section O, the American Society for Horticultural Science on Friday afternoon. Both sections of the physiological program were well attended.

The banquet was held in the Mark Twain Hotel on Friday evening, with President Paul J. Kramer presiding. Despite conflicts with dinners of other societies, the number attending the dinner for plant physiologists was greater than in pre-war years. Since no meeting had been held in 1945, two presidential addresses were given. Dr. B. S. Meyer, president of the society during 1943–44, presented a paper entitled "The Case for Greater Cooperation Among Plant Science Societies". Dr. H. A. Spoehr, the retiring president for 1944–45, spoke on "The Coming of Age of the American Society of Plant Physiologists". The Charles Reid Barnes Life Membership Award was made to Dr. D. R. Hoagland.

The Executive Committee held two meetings. The committee authorized publication of a new directory of members in the fall of 1946. The president was empowered to appoint two new committees, one on industrial relations and the second on the professional status of plant physiologists. Dr. R. Bouillenne of the Liege Botanical Institute, Belgium, was elected as a corresponding member. Storage of the archives of the Society at Miami University, Oxford, Ohio, was authorized. The Executive Committee approved action by the Society on the following constitutional changes, namely: that Article VIII, paragraph 2, sentence 2 be changed to read: "In case of a tie for fourth place, the secretary shall place the additional name or names on the ballot"; Article VIII, paragraph 4, be changed to read; "If two or more persons shall receive an equal number of votes for any office, the names of these persons shall receive an equal number of votes for any office, the names of these persons shall be resubmitted to the society"; Section 10 was amended to increase the Charles Reid Barnes Fund to \$4000.

The annual business meeting was held on Thursday afternoon. After acceptance of the officers' and committees' reports, it was voted by the attending members that the editor-in-chief be authorized to publish dates of receipt and final acceptance of papers published in *Plant Physiology*.

The report of the Executive-Secretary Treasurer indicated that the Society is in a sound financial condition. With total endowments of \$12,000, savings of \$5000, and cash on hand to the amount of \$8500, the Society should be able to carry on the usual activities even with rising costs

on all printing. The total membership is now 630 with subscriptions amounting to 575 in addition. An appeal is made for new members and subscriptions. The volume of printing reduces the cost of each succeeding copy, thus increasing the financial and professional strength of the society proportionately. Foreign memberships and subscriptions are rising slowly but steadily. Reserves of the journal make it possible to supply all missing back numbers.

**The Charles Reid Barnes Award.**—Professor Dennis Robert Hoagland of the University of California was awarded the Charles Reid Barnes Life Membership. Professor Hoagland was born in Golden, Colorado, on April 2, 1884. Upon graduation from Stanford University in 1907, he went to the University of California as instructor in Agricultural chemistry. Three years later he became chemist of the U. S. Department of Agriculture. After obtaining an advanced degree from the University of Wisconsin, he returned in 1913 to the University of California, where he has held professorships in agricultural chemistry and plant nutrition, and has been head of the division of plant nutrition since 1921.

Professor Hoagland soon became a leader in the field of the mineral nutrition of plants, and for the past thirty years his laboratory has continuously maintained its position among the foremost in the world. He has conducted a series of brilliant investigations on the nature of the soil solution, climatic influences on salt absorption, the accumulation of salts by plant cells, the dependence of accumulation upon metabolism, the role of the microtrophic elements, and the tracing of absorption and movement of salts by means of radio-active isotopes. Professor Hoagland is author of many scientific articles and of the recently published book entitled "Lectures on the Inorganic Nutrition of Plants."

Through his marked achievements in science, Professor Hoagland has earned many well deserved honors. He is a member of the National Academy of Sciences, and has served as president of the American Society of Plant Physiologists, of the Western Society of Soil Science, of the Western Society of Naturalists, and of the Pacific Division of the American Association for the Advancement of Science. He was the recipient of the first Stephen Hales Prize Award of the American Society of Plant Physiologists in 1929, and was joint recipient of the American Association Prize in 1940.

Professor Dennis Robert Hoagland, past-president of our Society, has been selected as the recipient this year of the Charles Reid Barnes Life Membership, in recognition of his researches on the mineral nutrition of plants. In behalf of the American Society of Plant Physiologists, the committee expresses its appreciation of Professor Hoagland's outstanding contributions to the plant sciences, and it extends to him best wishes for continued success in his field of scientific investigation.

**The New England Section.**—The New England Section of the American Society of Plant Physiologists will resume its schedule of annual meetings

May 17-18 at Harvard University, Cambridge, Massachusetts. Dr. Kenneth V. Thimann, Harvard University, is Chairman and Dr. Linus H. Jones, Massachusetts State College, is Secretary. The meeting, which will be held in the Harvard Biological Laboratories beginning on Friday afternoon, is open to all interested in the subject of plant physiology.

**E. C. Miller.**—The American Society of Plant Physiologists conveys its felicitations to DR. MILLER, on the occasion of his retirement after 35 years of active service in teaching and research. In recognition of his distinguished career as a scientist, the society presents a brief biographical record of DR. MILLER and his professional work.

DR. EDWIN CYRUS MILLER was born December 16, 1878, in a log cabin on a farm near Baltimore, Ohio. He received the B.S. degree in 1904 and the A.B. degree in 1906 from the old National Normal University at Lebanon, Ohio. Then followed an A.B. and the Ph.D. from Yale University in 1907 and 1910, respectively.

In the fall of 1910, DR. MILLER came to Kansas State College, where he was successively Instructor, Assistant Professor, Associate Professor, and, since 1919, Professor of Plant Physiology. He became a member of the Kansas Agricultural Experiment Station Staff in 1911 and has been Plant Physiologist of that Station since 1919. DR. MILLER served two years as acting head of the Department of Botany and Plant Pathology.

DR. MILLER continued to be active in the teaching of plant science at Kansas State College throughout his career, lecturing to students in the introductory course of plant science until two years before retirement and in the specialized courses in Plant Physiology until one year before retirement. One of his outstanding contributions to the teaching of plant science at Kansas State was his use of plants having economic value, plants for the most part known by the student, to illustrate his lectures and writings. He maintained an interest in the welfare of students and they were welcome to his counsel at all times. This interest in students was further manifested by the conscientious manner in which he carried out his assignment as a member of the College Committee dealing with failing students, an assignment which he held the last 15 of his 35 years at Kansas State College. His book, "Plant Physiology," which appeared in 1931 and the revised edition in 1938 has provided an outstanding text with extensive bibliographies for advanced classes, the graduate student, and the research worker.

DR. MILLER's research has had to do largely with the water relations and chemistry of plant life. His Ph.D. problem was a basic study of the changes and processes involved in the germination of a fatty seed (that of *Helianthus annuus*) which DR. WILLIAM CROCKER said in a review in the Botanical Gazette showed "both chemical and biological excellence." His first work at Kansas State College was the completing of two studies initiated during the progress of his doctoral problem at Yale. One had to do with the changes in the oily reserve of this seed at different stages in the development of the seedling, and the other with the origin of chloroplasts in its cotyledons.



DR. MILLER's first studies as a member of the Experiment Station staff were conducted at the Garden City Branch Station, and were concerned with the root systems of crop plants. They were technical studies with practical applications. They included a comparative study of the extent of root systems and leaf areas of corn and the sorghums, the relative water requirements of these plants, the daily variation of water and dry matter in their leaves, and their relative transpiration. These studies showed that the sorghum root system was better adapted to get water from the soil and while the sorghums lose more water per unit area of leaf surface, their total leaf surface was less than that of corn; thus is explained the observation of the farmer that sorghums withstand drought better than does corn. Then followed a study on the daily variation of the carbohydrates in the leaves of corn and the sorghums, and three studies in which DR. MILLER shared authorship: one having to do with the temperatures of leaves of crop plants; the second with the transpirational loss and water requirement of certain weeds and crop plants of Kansas; and the third, which has been widely cited, with the elemental composition of the corn plant. DR. MILLER's later station work was concerned with wheat of which his technical work on, "A Physiological Study of the Winter Wheat Plant at Different Stages of Its Development" was the most extensive. Two studies in which DR. MILLER was joint author were concerned with the relation of leaf rust infection to (a) yield, growth, and water economy and (b) diurnal transpiration in wheat. Three later studies on wheat conducted jointly with graduate students were concerned with (a) the influence of awns upon the rate of transpiration, (b) a consideration of the morphological nature and physiological functions of the awn, and (c) the effect of defoliation on the functions of winter wheat. DR. MILLER was author or co-author of 24 technical publications.

DR. MILLER served as President of the American Society of Plant Physiologists and as a member of the Editorial Board of that society. He is a member of Sigma Xi, Phi Beta Kappa, Phi Kappa Phi, A.A.A.S. (Fellow), Alpha Zeta, Gamma Sigma Delta, and Farm House Fraternity.

DR. MILLER has several hobbies from which he has derived diversion from scientific endeavors. One is a survey of the life and work of ABRAHAM LINCOLN which has resulted in a manuscript for a book entitled, "The Rise of Abraham Lincoln," a factual study of the life of this great American as seen through the eyes of a scientist. The second is a consideration of the importance of the corn plant in the life of man which has also resulted in a manuscript of book length. Both manuscripts are now being considered for publication. One of DR. MILLER's chief hobbies, aside from writing, was playing golf. His golf game, which he played for the fellowship involved rather than proficiency at the game, was well described in his droll statement, "I play brilliantly at times."

To know DR. MILLER intimately is to appreciate his many virtues which include straight-forwardness, a sense of fair play, a love of humor, friendliness, and high integrity. With the title Professor Emeritus of Plant Physi-

ology, DR. MILLER has retired to the farm on which he was born and which he now owns. Through his book on Plant Physiology he will continue to serve plant physiologists throughout the world. Almost one-fourth of the 6,000 copies sold to date (1945) have gone to students and libraries in foreign countries.

**National Research Council Representative.**—PROFESSOR B. S. MEYER of Ohio State University has been appointed representative of the American Society of Plant Physiologists on the National Research Council.

**Editorial Board of Plant Physiology.**—DR. F. P. CULLINAN of the Bureau of Plant Industry of the U. S. Department of Agriculture, Beltsville, Maryland, has been elected to a three year term on the Editorial Board of Plant Physiology by the Executive Committee of the American Society of Plant Physiologists.

**Biological Field Stations of the World.**—HOMER A. JACK. No. 1, Vol. 9, of *Chronica Botanica*. Chronica Botanica Co., Waltham, Massachusetts, and G. E. Stechert and Co., New York City. 73 pages. \$2.50.

A valuable addition to the *Chronica Botanica* collection, this booklet represents not only a coordination of the subject material existing in the literature, but also first-hand information on 79 biological stations in 18 countries which were visited by the author.

After a brief historical account of the location, administration, and functions of the various biological stations, they are generally described and compared. The second part of the book is organized as a directory of the Biological Stations arranged conveniently by countries. As much direct information as possible is included with each station, no matter how obscure; but of necessity, the descriptive accounts are corrected to 1940—before World War II became wide-spread. Several wood-cuts of biological stations are included.

**Organic Preparations.**—CONRAD WEYGAND. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 534 pages. \$6.00.

Available for the first time is this capable translation of Part II "Reaktionin" of WEYGAND's *Organisch-chemische Experimentierkunst*, published in Leipzig in 1938. Since all organic reactions consist of the breaking of existing bonds or the formation of new ones, the author has advantageously organized the chapters so that each is the discussion of the formation or cleavage of such bonds. The book advances from the simpler linkages to the more complex, and chapters are subdivided into the various reactions. A theoretical discussion under each heading precedes the laboratory instructions so that essentially the book combines the best features of a text and a laboratory manual for organic preparations.

The standard methods for preparations are footnoted extensively with references from the chemical literature. An extensive subject index is included in this fine, modern book.



# PLANT PHYSIOLOGY

JULY, 1946

## EFFECTS OF NITROGEN ON GROWTH AND ASH CONSTITUENTS OF *ANANAS COMOSUS* (L.) MERR.<sup>1</sup>

C. P. SIDERIS AND H. Y. YOUNG

(WITH EIGHT FIGURES)

### General

The preferred nitrogenous fertilizer in commercial plantations of pineapples is ammonium sulfate, according to JOHNSON (16). When sulfate of ammonia is used, the ammonium ion is oxidized to nitrate by soil microbiological activity, with an associated increase in soil acidity which renders iron more soluble. At the same time, both ammonium and nitrate ions are generally simultaneously available to the pineapple roots. Sodium nitrate is generally inferior and usually produces an atypical light-green color in leaves and unripe fruit of the pineapple. When sodium nitrate is used under dry conditions, however, there may be accumulation of sodium bicarbonate in the soil as reported by KELLEY and THOMAS (17) caused by a greater rate of uptake of nitrate than of sodium by roots and by soil microflora, a phenomenon likely to cause precipitation of iron in the soil and associated plant chlorosis. Efficient nitrogen fertilization of pineapple plants depends greatly on an understanding of the interrelationships of nitrate and ammonium ions with (a) the inorganic nutrient complex of the soil, that is, the relative concentrations of nutrient elements other than  $\text{NO}_3^-$  or  $\text{NH}_4^+$ ; (b) the activities of the microflora in the soil; and (c) shifting of pH values, and changes of temperature and moisture affecting the concentrations of these ions in the soil. It is also important to know the nitrogen requirements of plants at different growth stages on the basis of nitrogen inventories within the soil and plant tissues.

This paper reports plant weights attained by one-year-old *Ananas comosus* grown in solution cultures with 140.0 or 2.8 mg. of nitrogen in nitrate or ammonium form and the content of ash, water, relative electrical resistance, potassium, calcium, magnesium, phosphorus and iron in the tissue.

<sup>1</sup> Published with the approval of the Director as Technical Paper no. 164 of the Pineapple Research Institute, University of Hawaii.

## Cultural, chemical and statistical methods

Crowns, i.e., vegetative organs produced at the apical end of fruits, weighing approximately 100 gm. after sun curing and stripping of dried vestigial leaves, were suspended with bases in tap water for two weeks until roots developed. Plants with approximately 25 roots with an average length of 10 cm. were then selected in groups of 16 per culture and transferred to five-gallon porcelain crocks containing the nutrient solutions reported in table I. These solutions, constantly aerated and their acidity adjusted within a range of pH 4.5 to 6.5, were renewed at two-week intervals. Fourteen plants were removed from each nutrient solution after one year's growth

TABLE I  
COMPOSITION OF VARIOUS NUTRIENT SOLUTIONS

SALTS	SALT PER 100 LITERS (GRAMS)				ELE- MENTS	ELEMENTS PER MILLILITERS (MICROGRAMS)			
	SERIES					SERIES			
	NITRATE		AMMONIUM			NITRATE		AMMONIUM	
	HIGH- N*	LOW- N*	HIGH- N	LOW- N		HIGH- N	LOW- N	HIGH- N	LOW- N
	gm.	gm.	gm.	gm.		γ	γ	γ	γ
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	118.08	2.36	0.00	0.00	N	140.0	2.8	140.0	2.8
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.00	0.00	66.07	1.32	Ca	200.0	20.0	200.0	20.0
KH <sub>2</sub> PO <sub>4</sub>	6.81	6.81	6.81	6.81	K	48.8	48.8	48.8	48.8
K <sub>2</sub> SO <sub>4</sub>	6.53	6.53	6.53	6.53	P	15.5	15.5	15.5	15.5
CaCl <sub>2</sub>	0.00	10.10	56.00	11.20	S	18.1	18.1	178.1	21.3
MgSO <sub>4</sub> · 7H <sub>2</sub> O	24.65	24.65	24.65	24.65	Mg	12.2	12.2	12.1	12.2
FeSO <sub>4</sub> · 7H <sub>2</sub> O	1.39	1.39	1.39	1.39	Fe	2.7	2.7	2.7	2.7
ZnSO <sub>4</sub> · 7H <sub>2</sub> O	0.29	0.44	0.44	0.44	Zn	0.6	0.6	0.6	0.6
H <sub>3</sub> BO <sub>3</sub>	0.28	0.28	0.28	0.28	B	0.5	0.5	0.5	0.5
H <sub>2</sub> MoO <sub>4</sub> · H <sub>2</sub> O	0.02	0.02	0.02	0.02	Mo	0.1	0.1	0.1	0.1
MnSO <sub>4</sub> · 6H <sub>2</sub> O	0.26	0.26	0.26	0.26	Mn	0.5	0.5	0.5	0.5
					Cl	0.0	65.0	360.0	72.0

\* High-N = 140.0 mg. of nitrogen per liter.

Low-N = 2.8 mg. of nitrogen per liter.

and segregated into roots, leaves and stem. The leaves were further segregated into different age groups, and these and the stems were sectioned on the basis of differences in physiological functions in accordance with a technique reported elsewhere (30). Chemical methods for the analysis of the various constituents of the tissues have also appeared in former publications (27, 28, 29, 31, 32).

Calculation of the significance of the difference of the means of plant weights between different cultures was made by FISHER's method (10).

$$t = \frac{\bar{x} - \bar{x}^I}{s} \sqrt{\frac{(n_1 + 1)(n_2 + 1)}{n_1 + n_2 + 2}}$$

## Observations

## PLANT GROWTH

Table II shows that leaf and stem weights of the high-N cultures were

greater than of the low-N cultures, but root weights were relatively greater in the low-N than high-N cultures.

Ratios of weights of leaf, stem or root to plants for the various cultures are reported in table III and indicate that root weights in the low-N cultures gained at the expense of leaf and stem weights whereas in the high-N cultures the opposite condition obtained.

TABLE II

MEAN WEIGHTS OF TOTAL PLANTS, STEMS, LEAVES AND ROOTS OF ONE-YEAR-OLD PLANTS WITH CALCULATED "t" VALUES

SERIES OF	ITEMS COMPARED	N	MEAN WEIGHTS		CALCULATED "t" VALUES†
			HIGH-N*	LOW-N*	
			<i>gm.</i>	<i>gm.</i>	
Nitrate-N	Total plants	14	3650	2375	11.64
Ammonium-N	" "	14	3070	2605	2.60
Nitrate-N	Stems	14	376	147	6.00
Ammonium-N	" "	14	254	167	3.37
Nitrate-N	Leaves	14	2952	1755	26.30
Ammonium-N	" "	14	2660	2035	6.43
Nitrate-N	Roots	14	322	473	6.00
Ammonium-N	" "	14	156	403	13.72

\* High-N = 140.0 mg. of nitrogen per liter.

Low-N = 2.8 mg. of nitrogen per liter.

† All "t" values indicated highly significant differences between means (p less than 0.01) with the exception of value of 2.60, where significance level was slightly greater than p = 0.02.

The ratios of stem or root to plant weights indicate that in the high-N cultures, stems and roots but not leaves, comprised a greater proportion of the total plant in the nitrate than in the ammonium series.

#### WATER

The water content of tissues, reported in figure 1, was generally higher in the high-N than in the low-N cultures in both series in the basal (no. 1) and transitional (no. 2) sections of the mature (C) and active (D) leaves and in the roots. Also, in the ammonium series it was higher in similar sections of the young (E) leaves and in the stem. The water content of all other sections, except the basal stem sections of the high-N cultures in the nitrate series, was higher in the low-N than in the high-N cultures in both series.

TABLE III

RATIOS OF WEIGHTS OF LEAVES, STEMS OR ROOTS TO PLANTS IN THE VARIOUS CULTURES

ITEMS OF COMPARISON	NITRATE SERIES		AMMONIUM SERIES	
	HIGH-N	LOW-N	HIGH-N	LOW-N
Leaves to plants .....	0.810	0.739	0.866	0.782
Stems to plants .....	0.102	0.062	0.083	0.064
Roots to plants .....	0.088	0.199	0.051	0.154



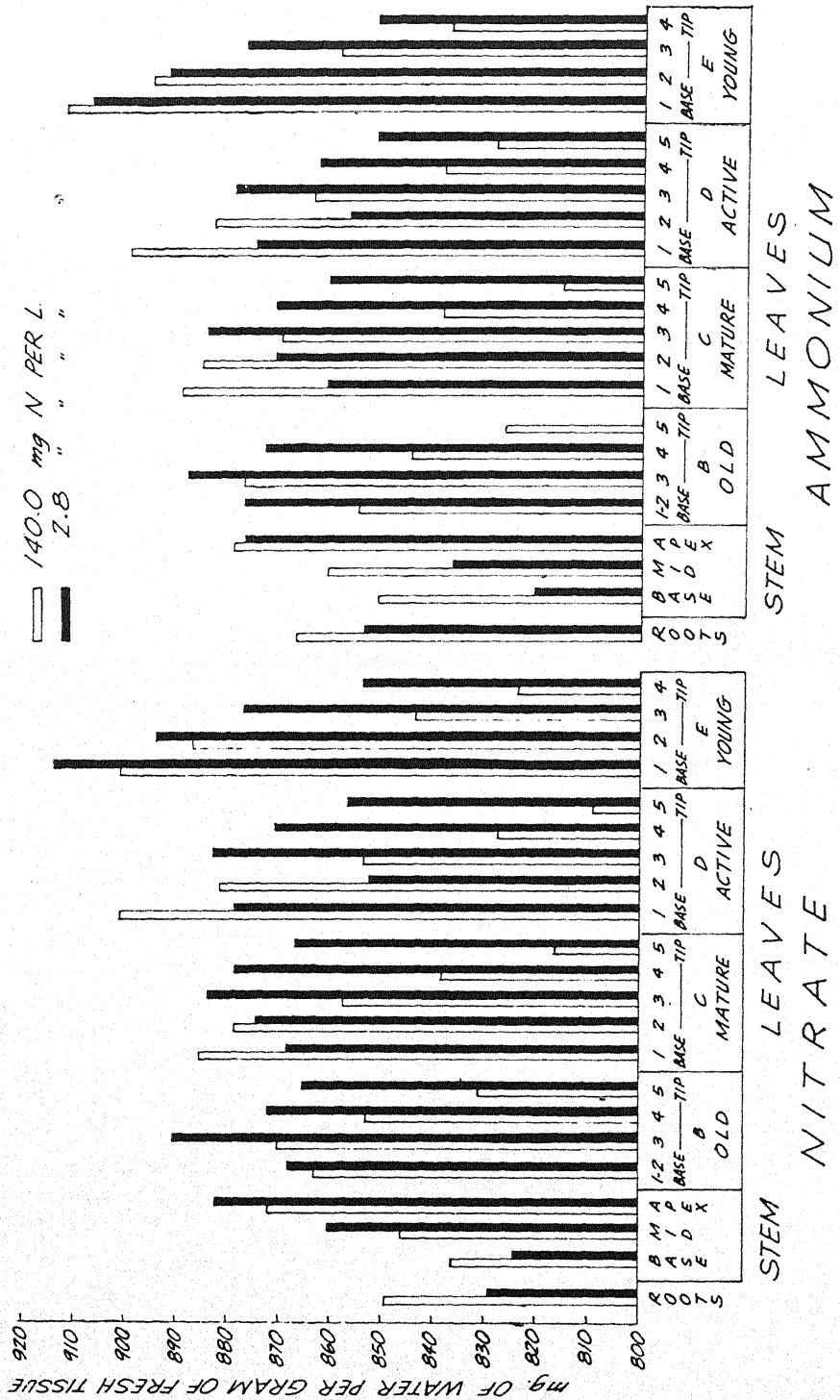


FIG. 1. Water in plant sections.

Differences in the moisture content of comparable sections between high-N and low-N cultures had resulted either from differences in the amounts of meristematic to differentiated tissues in the basal (no. 1) and transitional (no. 2) sections of the mature (C), active (D) and young (E) leaves, or from differences in the amounts of stored organic or inorganic solutes in the chlorophyllose (no. 3, 4 and 5) sections of the leaves. Similar differences in the moisture content of the stem may also be accounted for by the differences in the starch content.

#### ASH

Ash values, reported as mg. per gram of fresh tissue, in figure 2, were higher in the low-N than high-N cultures in all sections except the roots and the basal and medial stem sections of the nitrate series. However, the higher ash content of the low-N than high-N cultures in the nitrate series may be attributed to concentration effects which resulted from the lower plant weights in the former than in the latter cultures. Differences in the ash content of comparable sections between high-N and low-N cultures were generally greater in the ammonium than in the nitrate series. In the ammonium series the differences had possibly resulted from the antagonistic effects of high concentrations of  $\text{NH}_4$  ions in the high-N cultures on the absorption of other cations by roots, especially Ca ions, which effects were lacking in the high-N cultures of the nitrate series.

Total ash values per plant, reported in table IV, show that the high-N cultures in the nitrate series contained 40 per cent. more ash than the low-N cultures. However, in the ammonium series the ash content of the low-N cultures was 21.5 per cent. higher than that of the high-N cultures. The data further emphasize the fact that the low ash content of the high-N cultures in the ammonium series had resulted from a reduction of the rate of intake by roots of potassium, calcium and magnesium through the antagonistic effects of ammonium ions. In the nitrate series, although the ash content reported as mg. per gram of fresh tissue in figure 2 was higher in the low-N than high-N cultures, total ash values per plant, in table IV, were greater for the high-N than low-N cultures, indicating that the lower plant weights of the low-N cultures rather than a greater absorption of ash constituents raised the concentrations of the latter to higher levels in the low-N than high-N cultures.

#### ELECTRICAL RESISTANCE

Values of the relative electrical resistance (ohms) of the extracted sap, reported in figure 3, were indirectly proportional to the amounts of ash constituents. Factors directly affecting relative electrical resistance values are the concentration and degree of ionization of various inorganic and organic solutes in the sap, the latter not reported here because of their destruction during ashing. Electrical resistance values, being inversely proportional to the ionic content of the sap, were greater in the high-N than in the low-N cultures in conformity with the salt content in the tissues of these cultures, as reported in figure 2. For example, comparison of the data in figures 2

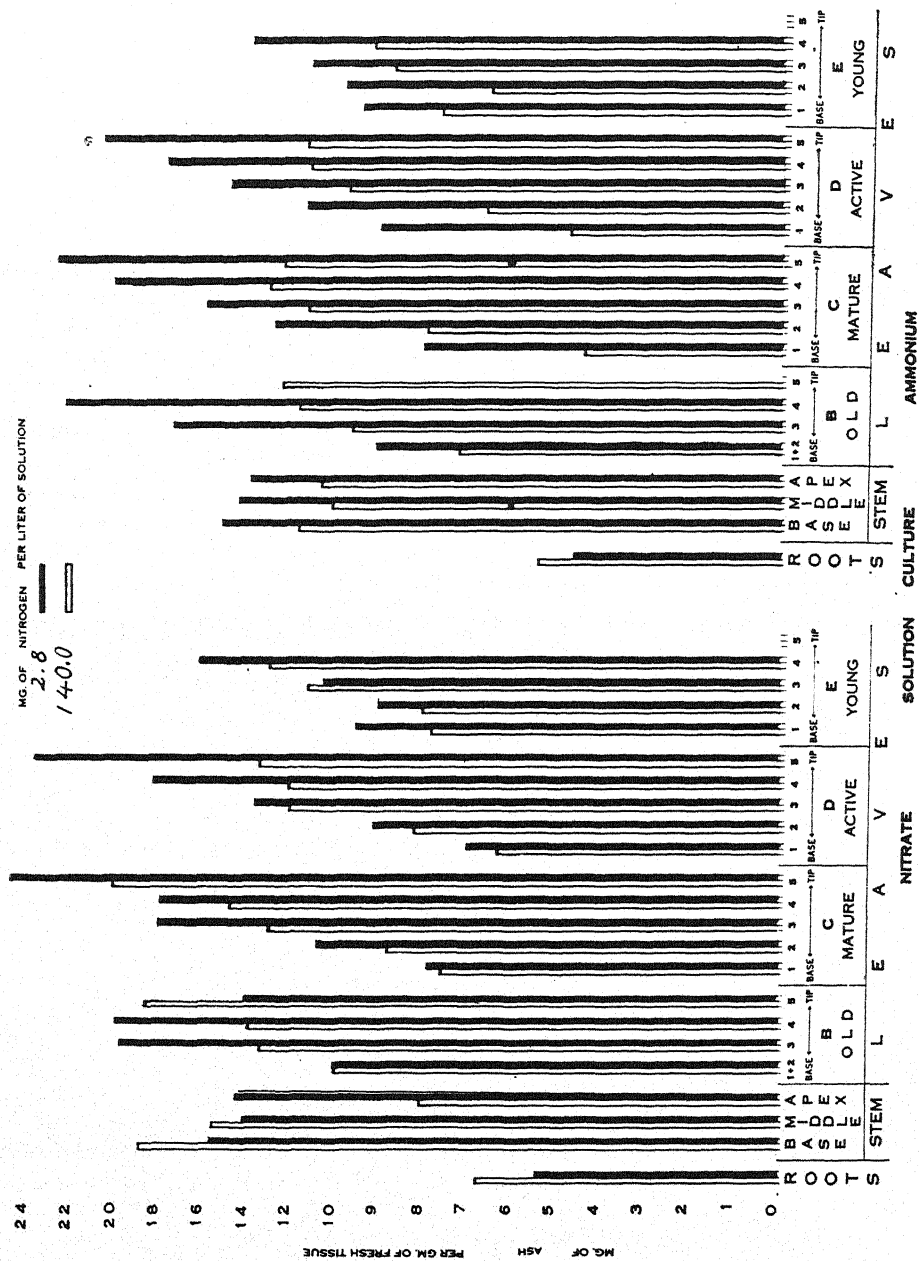


FIG. 2. Ash in plant sections.

TABLE IV  
AMOUNTS OF VARIOUS ASH CONSTITUENTS PER TOTAL PLANT, OR ORGANS OF *Ananas comosus*, GROWN IN  
HIGH-NITROGEN OR LOW-NITROGEN CULTURES

ITEMS		NITRATE SERIES										AMMONIUM SERIES									
		HIGH-N*					LOW-N*					HIGH-N					LOW-N				
		PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS				
ELEMENTS GM./PLANT	UNITS OF MEASURE	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.				
Ash	mg.	44,331	37,116	5,025	2,190	31,643	26,872	2,186	2,585	29,224	25,728	2,650	846	35,492	31,257	2,400	1,835				
K	"	18,787	17,032	1,069	686	17,588	15,548	744	1,296	13,282	11,921	1,063	298	25,522	22,527	1,375	1,620				
Ca	"	6,680	4,886	1,736	258	1,115	654	351	110	728	485	215	28	1,090	727	309	55				
Mg	"	1,039	886	92	61	1,056	791	103	162	967	720	196	51	1,424	1,171	155	99				
P	"	766	624	99	43	423	294	30	99	1,004	927	60	17	532	460	30	42				
Fe	γ	24,142	2,074	268	21,800	22,325	1,954	171	20,200	6,185	3,547	259	2,379	16,587	1,654	143	14,790				

\* High-N = 140.0 mg. of nitrogen per liter.

Low-N = 2.8 mg. of nitrogen per liter.



and 3 reveals that the tissues of the terminal leaf sections (no. 5), being chronologically more advanced than of other sections in the same leaves, contained more salt and had lower values for electrical resistance than the less advanced tissues in other sections. However, this rule did not apply to the meristematic tissues of the basal sections (no. 1) of the young (E) leaves which, because of a greater growth rate presumably possessed a higher degree of metabolic activity and required possibly greater salt concentrations than the chronologically more advanced tissues of adjacent sections with a reduced rate of growth.

#### POTASSIUM

Potassium, reported in figure 4 as mg. per gram of fresh tissue, was higher for the low-N than high-N cultures in both nitrogen series except for the terminal (no. 4) sections of the old (B) leaves in the nitrate series. Comparison of histogram heights in comparable cultures between the nitrate and ammonium series shows that potassium values for the high-N cultures were higher in the nitrate than ammonium series, whereas for the low-N cultures they were reversed. Total potassium values per plant, reported in table IV, were greater for the high-N than low-N cultures in the nitrate series, but in the ammonium series similar values were greater for the low-N than high-N cultures.

The data reveal that potassium absorption by roots from nutrient solutions was enhanced by the high content of  $\text{NO}_3$  ions in the high-N cultures of the nitrate series, but in the ammonium series it was greatly retarded by the high concentration of  $\text{NH}_4$  ions.

#### CALCIUM

Calcium values, reported in figure 5 as mg. per gram of fresh tissue, were greater for the high-N cultures in the nitrate series and the low-N cultures in the ammonium series than for the opponent cultures in either series. The effects of nitrate-ions enhancing calcium absorption from nutrient solutions by roots and of ammonium-ions retarding calcium absorption are clear cut.

Comparison of calcium values in the stem with those in the leaves shows that in the former organ they were many times greater than in the latter, possibly suggesting a higher rate of calcium absorption by the roots and transportation to stem than of translocation from stem to leaves.

Total calcium values per plant, reported in table IV, were higher for the high-N cultures in the nitrate series and for the low-N cultures in the ammonium series than for the low-N cultures in nitrate and high-N cultures in the ammonium series. The data on calcium, calculated either as mg. per gram of tissue or as total calcium per plant, indicated that  $\text{NO}_3$  ions in the nitrate series increased and  $\text{NH}_4$  ions in the ammonium series decreased the calcium content of tissues. Calcium differences between high-N and low-N cultures in the nitrate series, being greater than plant weight differences between the same cultures, indicate that the influence of high concentrations of  $\text{NO}_3$  ions in the high-N cultures enhancing calcium absorption was greater than the



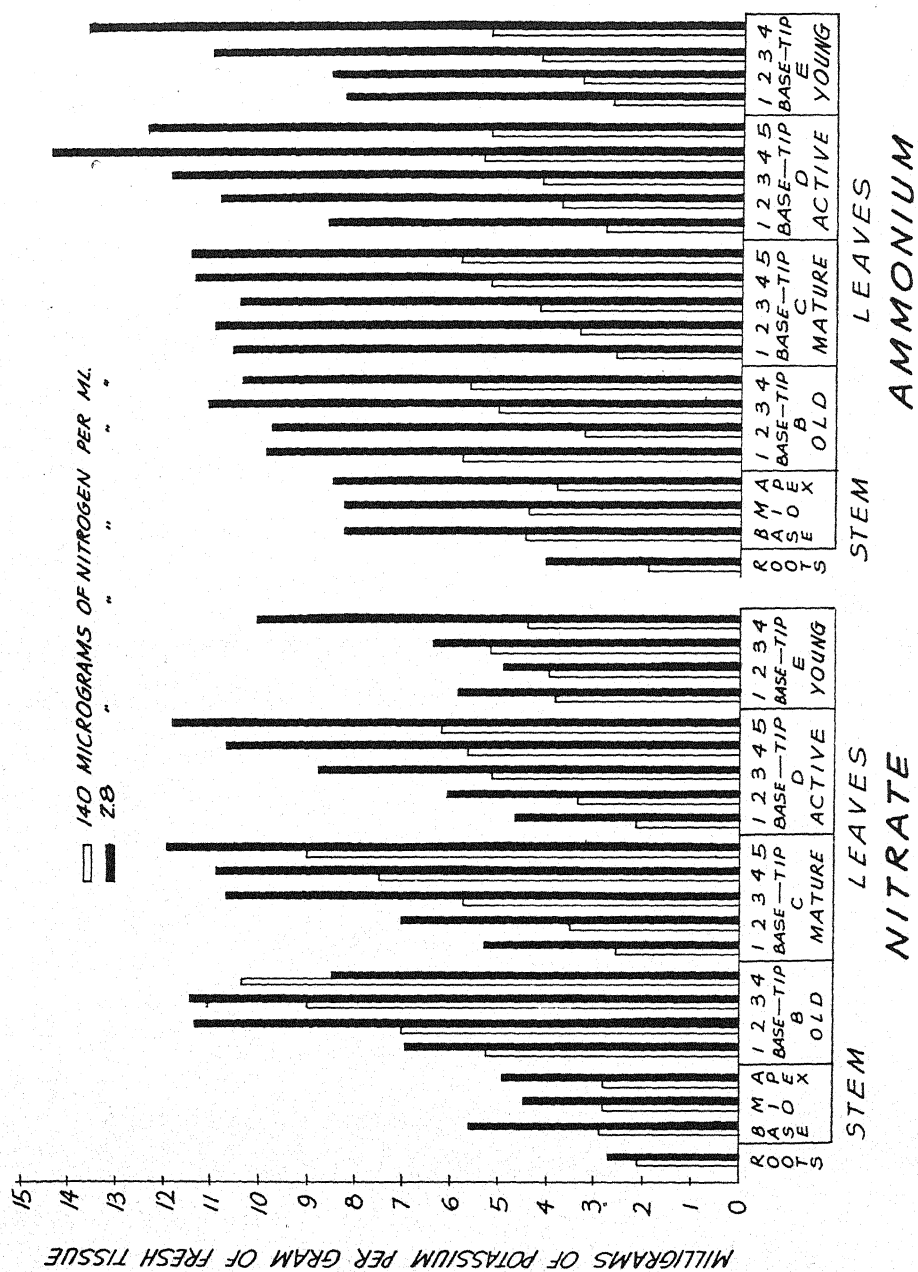


FIG. 4. Potassium in plant sections.

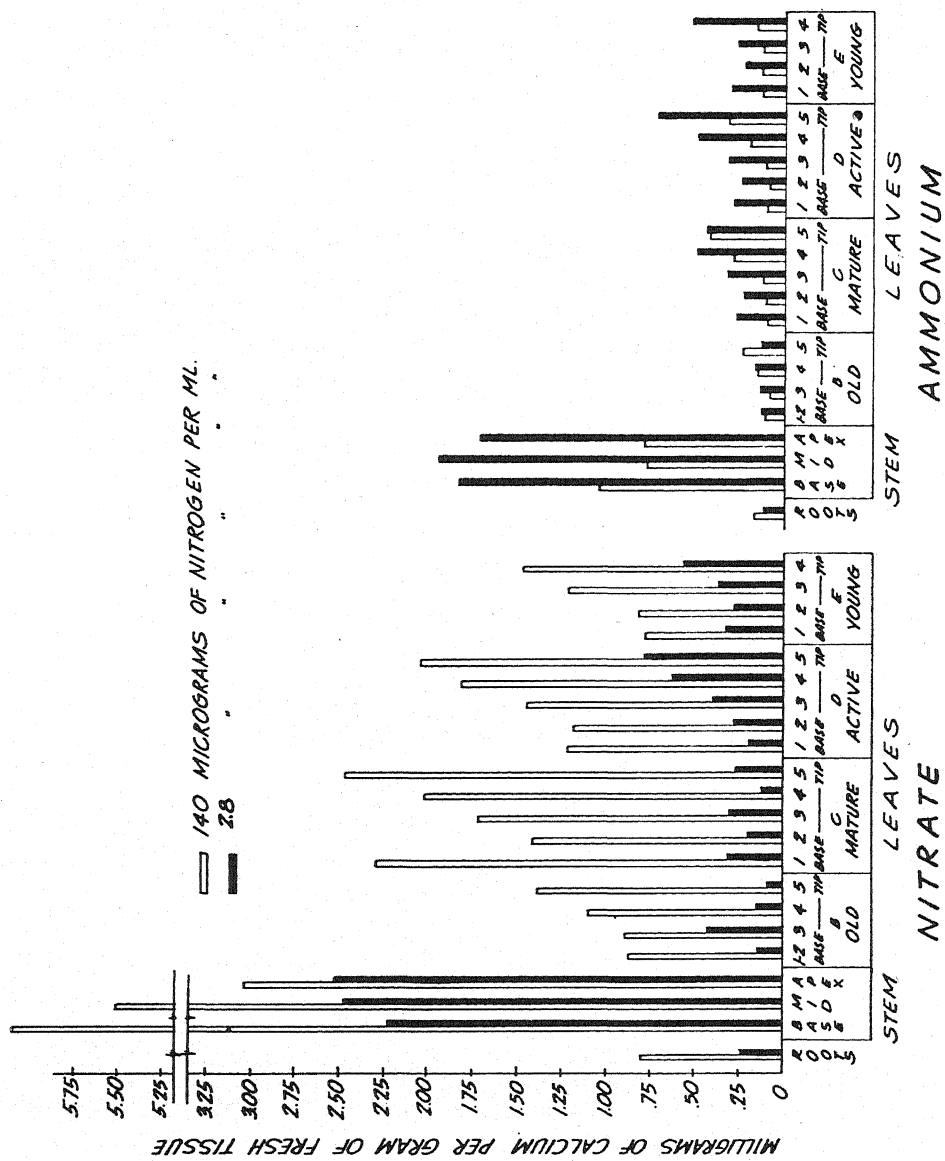


FIG. 5. Calcium in plant sections.

tendency for accumulation of calcium in the low-N cultures of the same series because of retarded growth and small plant volumes.

#### MAGNESIUM

Magnesium values, reported in figure 6 as mg. per gram of fresh tissue, were greater for the low-N than for the high-N cultures in both nitrogen series.

Total magnesium values per plant, in table IV, were approximately the same in the high-N and low-N cultures of the nitrate series, but in the ammonium series the low-N cultures contained approximately 47 per cent. more magnesium than the high-N cultures. It is possible that the difference in magnesium between high-N and low-N cultures in the ammonium series had resulted from a decreased rate of intake of Mg ions caused by the antagonistic effects of the high concentration of ammonium ions.

Accumulation of magnesium as percentage of total in the stem of the high-N cultures was 8.85 and 20.30 per cent. for nitrate and ammonium series, respectively, indicating a higher rate of absorption of magnesium and transport to the stem but a retarded rate of translocation from the stem to the leaves in the latter than former series. In the low-N cultures the differences in the magnesium content of stem and leaf tissues between nitrate and ammonium series were not as great as in the high-N cultures.

#### PHOSPHORUS

Phosphorus values, reported in figure 7 as mg. per gram of fresh tissue, were generally greater in the ammonium than in the nitrate series. Phosphorus differences between high-N and low-N cultures were in favor of the former cultures in both nitrogen series.

Total phosphorus values per plant, in table IV, were higher in the ammonium than nitrate series and were in agreement in this respect with the values in figure 7, suggesting that  $\text{PO}_4$  ions were attracted electrostatically by  $\text{NH}_4$  ions in the ammonium series and more so by the high-N than low-N cultures. However, comparable electrostatic repelling of  $\text{PO}_4$  ions by  $\text{NO}_3$  ions could not be observed in the high-N cultures of the nitrate series because these cultures showed 1.8 times as great intake of  $\text{PO}_4$  ions as the low-N cultures of the same series. Phosphate concentrations in the nutrient solutions being relatively small, the conditions for the phenomena of antagonism were probably not propitious.

#### IRON

The data in figure 8 on the iron content of the various cultures are more involved and less suitable to interpretation than those pertaining to other nutrient elements because of the high susceptibility of iron to precipitation on the epidermal cells of roots during adsorption and its low translocation rate from roots to other plant organs. Such precipitation on the epidermal root layer is greatly influenced by changes in the hydrogen-ion concentration at the interfacial layer, resulting from an unequal absorption of anions and cations.

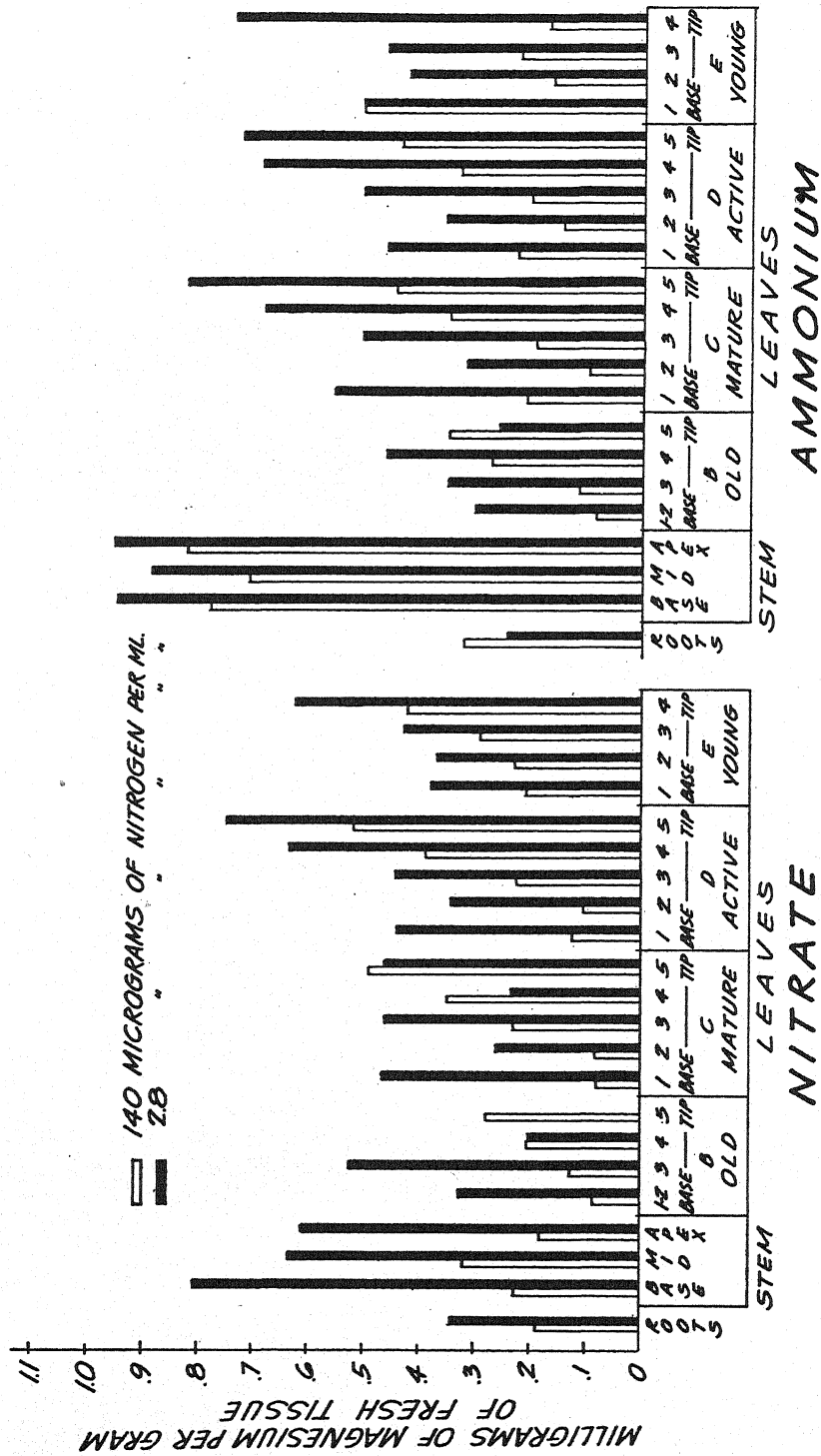


FIG. 6. Magnesium in plant sections.



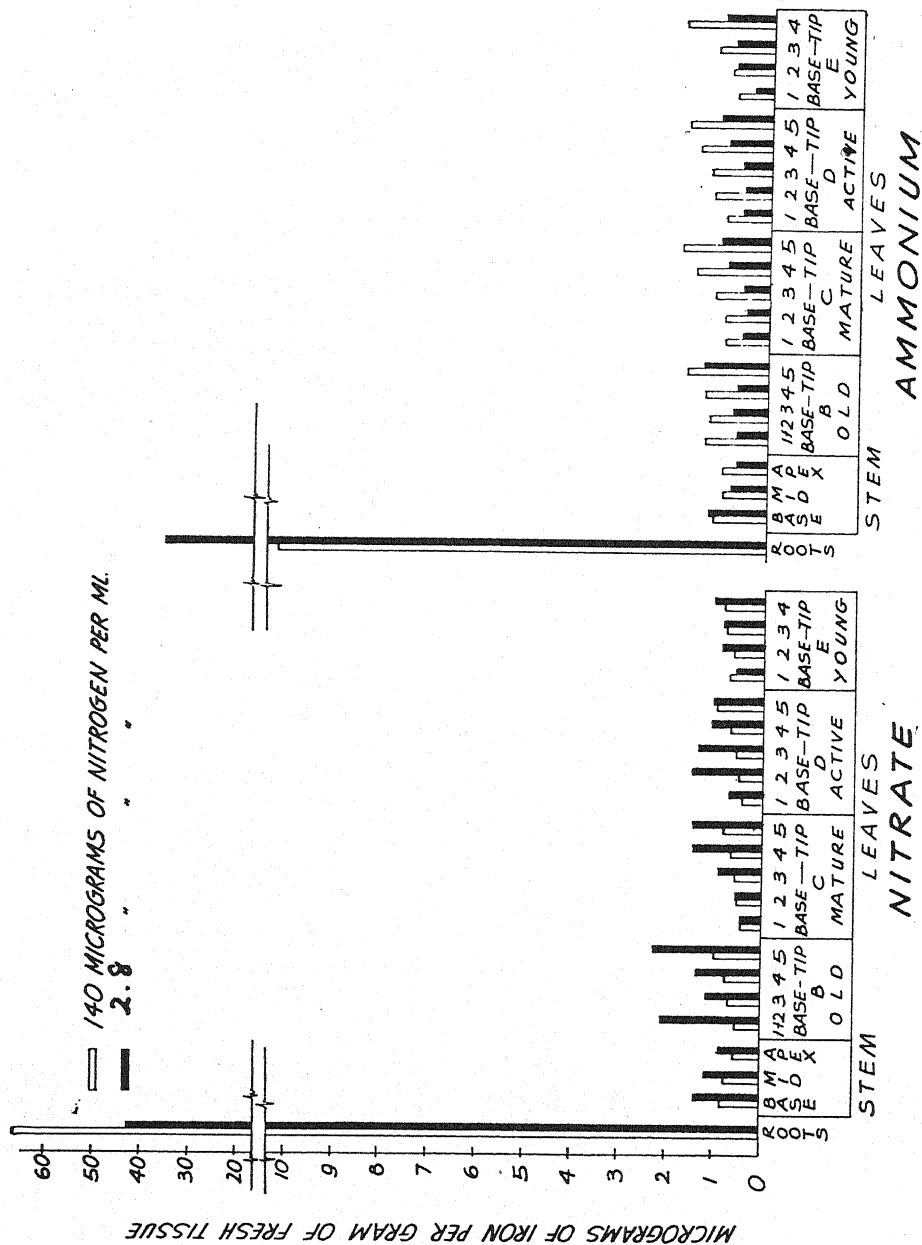


FIG. 8. Iron in plant sections.



Comparison of iron concentrations in the root tissues with the mean iron concentration of all other plant sections shows that in the nitrate series the former were 98.3 and 37.5 times as high as the latter for the high-N and low-N cultures, respectively. In the ammonium series corresponding ratios were 12.1 for the high-N and 47.2 for the low-N cultures. The data reveal that iron precipitation on the epidermal root cells was greatest for the high-N cultures in the nitrate series and smallest for the high-N cultures in the ammonium series. Iron precipitation on the roots of the low-N cultures in both series was intermediate between the high-N cultures of the nitrate series on the one side and ammonium series on the other side. The high iron precipitation in the nitrate series had resulted from the shifting of pH from 4.5 to 6.6 because of greater absorption from the nutrient solution of nitrate than calcium ions; whereas low iron precipitation on the roots of the high-N cultures in the ammonium series had resulted from a countershift in the pH course, e.g., from 6.5 to 4.0 caused by a greater absorption of  $\text{NH}_4^+$  than  $\text{SO}_4^-$  ions and formation of  $\text{H}^+$  ions by hydrolysis. Average iron concentrations in the plant, excluding the roots, were greatest in the high-N cultures of the ammonium series and smallest in the high-N cultures of the nitrate series. Comparison of iron concentrations in the roots with the average concentrations in the combined leaf and stem sections shows that in the latter organs iron concentrations were inversely proportional to the amounts of iron precipitated on the roots.

Total iron values per plant, reported in table IV, reveal very little unless considered with respect to its distribution in the various organs. The iron content of the leaves of the high-N cultures in the ammonium series was 1.71 times as great as that of the high-N cultures in the nitrate series. Iron in the leaves of the low-N cultures in the nitrate series was 1.19 times as great as that for comparable cultures in the ammonium series. Differences in the iron content of the stems were smaller between comparable cultures in different series than between different cultures in the same series; for example, the difference in stem iron between the nitrate and ammonium cultures was 3.5 per cent. for the high-N cultures and 19.6 per cent. for the low-N cultures. But, similar differences between high-N and low-N cultures in the same series were 56.7 and 81.2 per cent. in favor of the former cultures for the nitrate and ammonium series, respectively. Differences in the total iron of the roots between the high-N cultures in the nitrate and ammonium series were 816.0 per cent. in favor of the former series. Similar differences between the low-N cultures were 36.5 per cent. in favor of the nitrate series. Differences in total root iron between high-N and low-N cultures in the nitrate series were 7.9 per cent. in favor of the high-N cultures, but similar differences in the ammonium series were 522.0 per cent. in favor of the low-N cultures. The amounts of iron deposited or precipitated in the roots of the high-N cultures (as the result of H-ion changes, mentioned above) were 9.15 times greater for the nitrate than ammonium series, whereas the amounts of iron transported to the leaves were 1.7 times greater for the ammonium than

nitrate series. These results clearly indicate that the greater iron depositions in the roots of the nitrate cultures constituted insoluble iron which was unavailable for translocation to the tissues of the stem and leaves, whereas the smaller iron depositions in the roots of the ammonium series suggest a greater availability for translocation to other organs of the plant. The above results are in agreement with other findings on the effects of  $\text{NO}_3$  and  $\text{NH}_4$  ions in connection with other studies reported previously (32, 33).

### Discussion

Synoptic review of the results obtained shows that a fifty-fold increase in the external concentration of nitrogen, i.e., from 2.8 to 140.0 mg. per liter, increased the total nitrogen uptake by plants 4.85 and 5.35 times in the nitrate and ammonium series, respectively. Plant weights were increased 1.537 and 1.180 times in the nitrate and ammonium cultures, respectively. Comparative plant weight increases between the high-N and low-N cultures were lower for the ammonium than nitrate series which should be attributed to the growth inhibiting properties of the chloride content of the former series. Calcium uptake by plants from the nutrient solution was 6.00 times as great for the high-N as low-N cultures in the nitrate series and 0.67 times as great for the high-N as low-N cultures in the ammonium series. Potassium absorption was approximately the same for the high-N and low-N cultures in the nitrate series and magnesium showed the same relationship. In the ammonium series, however, the high-N cultures absorbed 0.52 times as much potassium and 0.68 times as much magnesium as the low-N cultures. Phosphate uptake was 1.81 and 1.88 times greater by the high-N than low-N cultures of the nitrate and ammonium series, respectively. Iron uptake, by the high-N cultures, as determined by total iron in leaves and stem being greater 1.11 and 2.11 times for the nitrate and ammonium series, respectively, was influenced more by changes in the H-ion concentration resulting from the uptake by roots of anions and cations at unequal rates than by the direct effects of different concentrations of nitrate or ammonium ions.

The results as stated above appear to be in satisfactory agreement with results obtained by various investigators with other plants. EATON and RIGLER (9) observed in the cotton plant that an increase in nitrate from a low level resulted in an increase in vegetative growth, in an increased number of bolls, and in higher concentrations of nitrates and total nitrogen in the plant tissues. WADLEIGH (35) working also with cotton found that increasing the nitrate content of the substratum from 8 to 25, 75, and 225 p.p.m. increased plant nitrate nitrogen from 0.08 to 0.225, 0.80, and 1.38 gm. and yield of seed from 27.0 to 58.1, 119.8, and 143.0 gm. per plant, respectively. Somewhat similar results were obtained by BENSEND (6) with different amounts of nitrogen on Jack pine, *Pinus banksiana* Lamb., seedlings which gained sixteenfold in weight as the amounts of nitrogen were increased from 0.0 to 230 p.p.m., but higher increases to 855 p.p.m. showed slight weight reductions. BECKENBACH *et al.* (3, 4, 5) observed in corn (*Zea mays*) that

plant weights, nitrate concentrations and total nitrogen in the tissues increased with higher amounts of nitrates in the substratum. BARTHOLOMEW *et al.* (2) observed that tomato plants grown in nutrient solutions with an abundant supply of nitrogen and potassium produced greater yields of dry matter and had a higher percentage of nitrogen in the leaves than those supplied with low nitrogen.

Absorption of nitrate nitrogen and other anions is associated with proportional energy expenditure, according to LUNDEGARDH (19) which is indicated as moles of respired  $\text{CO}_2$  per mole of  $\text{NO}_3^-$  or of other anions. His contention is that roots, being negatively charged, absorb metallic cations by attraction; whereas anions are repelled from the negative surface and an extra supply of energy, provided by carbohydrate oxidation in respiration, is needed to overcome the resistance. However, this view of LUNDEGARDH has been challenged by STEWARD (34) and HOAGLAND and STEWARD (15), who claim that their experiments on barley roots and on disks of potatoes have not led to a fundamental distinction between cation and anion accumulation; moreover, the cation ammonium and the anion nitrate usually produced the most marked acceleration of respiration over that occurring in distilled water. GREGORY (12) claims that nitrogen deficiency in barley always leads to a reduction in the rate of respiration. McCALLA and WOODFORD (22) state that when nitrogen was limited more phosphorus and sulphur were taken in by wheat plants than when nitrogen levels were high. ROSE and McCALLA (25) found that limiting nitrogen reduced the size of plants and the amounts of all nutrients absorbed except phosphorus. SHANK (26) observed that top to root ratios increased with an increase in nitrogen concentration, and he attributes this condition to the failure of translocation to leaves of substances absorbed by roots because of insufficiency for optimal growth.

ARNON (1), discussing the merits of nitrate *vs.* ammonium sources in the nutrition of barley and obtaining a more satisfactory growth with the former than latter, claims that "furnishing the plant with nitrogen exclusively in the reduced form of ammonium may be regarded as not merely a substitution of one source of nitrogen for another but a deprivation of the plant of an oxidizing agent." That nitrates might act in the capacity of oxidizing agents is indicated by MEYERHOF's computations (21) of WARBURG and NEGELEIN's (36) results which show that, of the energy released by the oxidation of carbon to  $\text{CO}_2$ , only 30 per cent. is used in the reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$ . HAMNER (13) found that wheat plants supplied with nitrate assimilated approximately 56.0 per cent. and liberated by their roots 27.0 and tops 66.0 per cent. more carbon dioxide than similar plants grown in minus-nitrate cultures. However, in the absence of similar cultures with ammonium cations, one is unable to attribute the gain in respiration to the nitrate ions alone or to an increased metabolic activity resulting from a greater protoplasmic volume by the addition of nitrogen to the cultures. Recent studies by GILBERT and SHIVE (11) have indicated that production

of  $\text{CO}_2$  by roots was increased with a greater nitrate uptake but not with ammonium.

The utilization of oxygen released by nitrate reduction to ammonium for respiration by roots, mentioned above, has not been investigated, although in *A. comosus* the various phases of nitrate assimilation taking place mostly in the chlorophyllose regions of the leaves (30) suggest that any oxygen released by reduction of nitrates might be utilized by the adjacent chlorophyllose tissues or may be transported downward to the roots in solution with other organic solutes.

Comparison of these results with those of the various investigators, above mentioned, shows that a very satisfactory agreement was observed with respect to nitrogen supplies in the nutrient solution and plant weights, intake of total nitrogen in both nitrate and ammonium series, and potassium and calcium in the nitrate series. Total phosphorus absorption was greater in the high-N than low-N cultures of the nitrate series, which was not in agreement with the results of McCALLA and WOODFORD (22) and ROSE and McCALLA (25). The differences in pH shifts of nutrient solutions supplied with nitrate or ammonium salts have been discussed with respect to iron solubility and availability to plants. BRIGGS (7) observed in *Tropaeolum majus* that boron in small amounts may greatly enhance nitrate intake by roots.

The relations between cation and anion accumulation in plant tissues have been discussed by HOAGLAND and BROYER (14) who state that "in a number of experiments on root tissues possessing a high potentiality for salt absorption, K and Br were withdrawn from the solution in nearly equivalent quantities, although various secondary effects may complicate the study of this relation by causing K losses from cells, etc." The same authors, commenting on nitrate absorption and accumulation, state that "the concentration of  $\text{NO}_3$  in the sap increased very rapidly during the period of 16 hours then fell off abruptly, presumably the rate of  $\text{NO}_3$  reduction in the tissues greatly exceeded the rate of  $\text{NO}_3$  accumulation." LUNDEGARDH (18, 20), discussing the rôle of cations on the electrostatic charges of cell membranes which influence anion attraction, claims that "bivalent ions, such as  $\text{Ca}^{++}$  and  $\text{Mn}^{++}$ , attract water molecules from the surroundings and consequently dehydrate the membrane, causing the molecules to pack closer together and increase the charge of the membrane, which is a function of the number of valences per unit of area." Regarding potassium, he states that "a membrane in which the free valences are chiefly saturated with  $\text{K}^+$  has a looser constitution, the electrical charge is lower and the membrane probably does not resist high charges." Further evidence on the nature of the negative charge of plant cells is offered by OSTERHOUT (23), who states that "remarkable changes are brought about by dilute solutions of KOH but not as effectively by NaOH in transforming the negative cells of *Nitella* to positive cells by dissolving out a fatty acid, a constituent of the protoplasm charged negatively." Moreover, the same author (23) claims, on the basis of ionic

mobilities, that "the protoplasmic surface cannot be a pore system, for in such a system all cations must have greater mobilities than all anions or vice versa." CONWAY (8), attempting to explain the differential rate of ion penetration through membranes as being due to different diameters of the ions, gives the following relative diameters referred to K-ion as unity: K = 1.00; Na = 1.49; Ca = 2.51; and Mg = 2.81. The relative diameters of the ions mentioned by CONWAY are different from the ionic radii of the same elements of PAULING (24) with values in Å as follows: K = 1.33; Mg = 0.65; Ca = 0.99; NO<sub>3</sub> = 1.21, etc. The data in table V indicate a ratio of potassium

TABLE V  
AMOUNTS OF ELEMENTS PER PLANT AND RATIOS OF ELEMENTS ABSORBED

ELEMENTS GM./PLANT	NITROGEN				POTASSIUM†			
	N-N		A-N		N-N		A-N	
	HIGH-N*	LOW-N*	HIGH-N	LOW-N	HIGH-K	LOW-K	HIGH-K	LOW-K
K	18.79	17.59	13.28	25.52	43.55	5.97	39.44	7.13
Ca	6.68	1.12	0.73	1.09	1.78	4.00	1.18	1.05
Mg	1.04	1.05	0.97	1.42	0.70	1.42	0.88	1.19
N	8.64	1.78	9.76	1.82	5.99	4.68	5.82	5.78
P	0.77	0.42	1.01	0.53	0.53	0.25	0.64	0.31
Ratios								
K/Ca	2.81	15.70	18.20	23.40	24.50	1.50	33.50	6.80
K/N	2.19	9.88	1.36	14.00	7.27	1.28	6.78	1.23
Ca/N	0.77	0.63	0.08	0.60	0.30	0.86	0.20	0.18

\* High-N and K = 140.0 mg. N and 200 mg. K per liter, respectively.

Low-N and K = 2.8 gm. N and 4 mg. K per liter, respectively.

† Reference (33).

to calcium of 2.81 (18,787 ÷ 6,680), for the high-N cultures in the nitrate series, differing from the theoretical ratio of ionic diameters (2.51) proposed by CONWAY, 12.0 per cent. The ratio of K to Mg for the same culture and series was 17.3 : 1 (18,787 ÷ 1,039) which is 509 per cent. greater than that postulated by the diameter ratios of K<sup>+</sup> to Mg<sup>++</sup> ions. Similar ratios for elements absorbed from the low-N cultures in the nitrate series or in the ammonium series were not in agreement with the diameter ratios of the ions, possibly because the amounts of NO<sub>3</sub><sup>-</sup> ions in the low-N cultures were abnormally low with respect to the various cations, or the antagonistic effects of NH<sub>4</sub><sup>+</sup> ions in the ammonium series interfered with cation absorption. Further evidence of ion interrelationships, indicated by the total amounts of certain nutrient elements in the tissues of various cultures, is presented in table IV, which shows that ratio values of potassium to calcium were not in agreement with the theoretical relative diameter 2.51 except for the high-N culture in the N-n series. Potassium to nitrogen ratios also deviated appreciably from the equimolecular value of 2.785 (39 ÷ 14), being high in cultures with too little nitrogen or too much potassium and low in those with too much nitrogen or too little potassium. Calcium to nitrogen ratios show that the high ammonium content of the high-N culture in the A-n series, and also a similar



content of potassium ions of the high-K culture in the same series depressed greatly calcium absorption. Phosphorus values in the tissues were almost twice as great for the high-N or high-K than for the low-N or low-K cultures, indicating that high concentrations of  $\text{NH}_4$  ion enhanced intake of  $\text{PO}_4$  ions and also that similar concentrations of  $\text{NO}_3$  ions had not interfered with the absorption of  $\text{PO}_4$  ions from solution culture.

Comparison of the data under nitrogen and potassium (33) in table V shows that the chemical composition of plant tissues in mineral nutrient elements is not constant but varies widely and depends on the amounts of such elements taken in by the roots. High concentrations of  $\text{NH}_4$  or K cations reduce the intake of Ca cations and enhance that of  $\text{PO}_4$  anions.

### Summary

1. *A. comosus* grown in solution cultures supplied with 140.0 or 2.8 mg. of nitrogen per liter either as nitrate or ammonium produced, after one year's growth, greater weights in the high- than low-nitrogen cultures. Nitrogen absorption from nutrient solutions was approximately five times greater for the high-nitrogen (140.0 mg.) than low-nitrogen (2.8 mg.) cultures in both series.

2. Total ash content per plant was higher in the high-nitrogen cultures of the nitrate series and in the low-nitrogen cultures of the ammonium series than in the competing cultures, possibly because of  $\text{NO}_3$  anions attracting cations and  $\text{NH}_4$  cations repelling similar cations in the nitrate and ammonium series, respectively.

3. Potassium values per plant were approximately the same for the high-nitrogen and low-nitrogen cultures in the nitrate series, but in the ammonium series they were approximately 92.0 per cent. greater for the low-nitrogen cultures; presumably high  $\text{NH}_4$  ion concentrations caused inhibition of K-ion intake.

4. Calcium values per plant were greater for the high- than low-nitrogen cultures in the nitrate series. In the ammonium series calcium values for the high-nitrogen cultures were approximately two-thirds as great as in the low-nitrogen cultures, resulting, presumably, from the antagonistic effects of high concentrations of  $\text{NH}_4$  ions in the high nitrogen cultures.

5. Magnesium absorption per plant from nutrient solutions was approximately the same for the high- and low-nitrogen cultures in the nitrate series, but in the ammonium series it was 1.475 times as great for the low- as for the high-nitrogen cultures.

6. Phosphorus content per plant was 1.81 and 1.72 times greater for the high- than low-nitrogen cultures in the nitrate and ammonium series, respectively.

7. Iron content per plant was greater for the cultures in the nitrate than ammonium series, but in the former series 90.3 per cent. of it was in the roots whereas, in the latter series, the roots of the high-nitrogen cultures contained 38.5 per cent. of total plant iron and those of the low-nitrogen cultures



89.5 per cent. Translocated iron in the leaves as percentage of total iron was 8.6 or 8.8 in the nitrate series and 57.5 or 10.0 per cent. in the ammonium series for the high-nitrogen or low-nitrogen cultures, respectively.

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# NITROGEN METABOLISM OF CORN (*ZEA MAYS*) AS INFLUENCED BY AMMONIUM NUTRITION<sup>1</sup>

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(WITH FOUR FIGURES)

In a previous publication (45) data were presented to show that large amounts of glutamine, asparagine, amino acids, and other forms of soluble nitrogen, accumulated in the sap of the tops of corn seedlings in response to the absorption of large amounts of ammonium nitrogen from culture solutions. Ammonia did not accumulate in the sap and visible injury did not result until the soluble forms of organic nitrogen had reached relatively high levels. The pH of the sap, the total and reducing sugars, and the "true protein" nitrogen remained relatively stable during the period of ammonium absorption. It is the purpose of this paper to present data for the roots and tops in greater detail showing the probable site of the metabolism of the absorbed ammonium and further information on the nature of the soluble nitrogen compounds synthesized.

No attempt will be made to review the extensive literature on the nitrogen metabolism of plants since several excellent reviews are available (2, 20, 46), but attention will be confined to that dealing with the nitrogen nutrition of corn insofar as it is related to this investigation. In spite of the great economic importance of corn surprisingly little has been reported concerning its nitrogen metabolism. As early as 1868 BOUSSINGAULT (cited by CHIBNALL (2)) reported that asparagine was formed in corn germinated in the dark. More modern reports are those of PRIANISCHNIKOW (26) who showed the necessity of carbohydrates for the formation of asparagine in corn and JODIDI (13) who crystallized asparagine from etiolated corn seedlings. KLEIN and TAUBOCK (16) demonstrated that corn in sterile culture solutions would absorb arginine and would decompose it completely within the plant without urea being one of the products that accumulated. Alkaloids are rarely found in the monocotyledons. Hydrocyanic acid and cyanogenic glycosides rarely occur in corn and when present occur only in traces according to a review of the literature by VAN DER WALT (36). Tests at various times in this laboratory on maturing corn have failed to indicate even traces of cyanide nitrogen.

MEVIUS (19) found that corn supplied with ammonium salts grew well if the culture solution pH was maintained between 3.5 to 7.0, but that above this range the internal reaction became alkaline, excessive quantities of ammonia were absorbed, and growth was depressed.

<sup>1</sup> Published with the approval of the Director of the South Dakota Agricultural Experiment Station as Journal Paper no. 201.

## Materials and methods

## CULTURE TECHNIQUE

The plan was to grow corn seedlings to a height of about 18 inches in dilute nutrient solution, depriving them of nitrogen during the later stages of growth to produce plants low in soluble nitrogen and high in carbohydrates, and then to transfer them to a complete nutrient solution containing the nitrogen as  $(\text{NH}_4)_2\text{SO}_4$ , harvesting plants at various intervals for subsequent analyses. Ammonium nitrogen was used to avoid problems of nitrate reduction and nitrate accumulation that would be involved if nitrate nitrogen were used.

The first experiment was conducted during December and January with greenhouse temperatures of  $65^\circ$  to  $75^\circ$  F., and the second in February and March with temperatures varying from  $70^\circ$  to  $90^\circ$  F. Supplementary light of moderate intensity was used from 4 to 9 A.M. in both experiments to insure a high carbohydrate level. South Dakota 105  $\times$  107 single cross hybrid seed was germinated in pure quartz sand and uniform plants were transplanted, when they were 4 inches high, to corks in special lids which fit loosely on 2-gallon glazed earthenware crocks.

For the first experiment the plants were grown, 3 per crock, in 14 crocks, each containing 7 liters of one-half strength Hoagland solution supplemented with a microelement solution containing Cu, Mo, Mn, Zn and Bo [(11) p. 37]. Iron was added semi-weekly as  $\text{FeSO}_4$  to all culture solutions used. When the plants were about 18 inches tall the solution was replaced with a minus-nitrogen solution of the following composition:

0.5 M $\text{K}_2\text{SO}_4$ .....	5 ml./liter
1.0 M $\text{MgSO}_4$ .....	2 ml./liter
0.05 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$ .....	10 ml./liter
0.01 M $\text{CaSO}_4$ .....	200 ml./liter

Micronutrients were also added to this solution. The pH of the solution remained at approximately 6.0 during the growth of the plants. After 1 week the lower leaves, particularly along the midribs, were chlorotic indicating nitrogen deficiency and the soluble nitrogen of the tops and the roots had been reduced to a low level as indicated by subsequent analyses of 6 plants harvested as a control (0 days, table I). To the solutions in the remaining crocks molar  $(\text{NH}_4)_2\text{SO}_4$  was added to give the concentrations indicated in table I. After 3 days, 4 more crocks (12 plants) selected at random were harvested, and more  $(\text{NH}_4)_2\text{SO}_4$  was added to the remaining solutions. This procedure was continued until all plants were harvested to give the series shown in table I. The pH of the solutions remained at  $6.5 \pm 0.1$  during this period of ammonium absorption.

In the second experiment, conducted to check the results of the first and to supply material for a more detailed study of the nitrogen compounds synthesized, the technique was similar except that 5 plants were grown in

each crock containing 7 liters of Hoagland solution for the preliminary growth period. After the period of nitrogen depletion in minus-nitrogen solutions, 15 plants were harvested as a control, 60 meq./l. of  $(\text{NH}_4)_2\text{SO}_4$  were added to the remaining solutions, and after 7 days, 15 plants were harvested. In this experiment the pH of the culture solution during the period of ammonium absorption had to be frequently adjusted with 1 N NaOH to maintain the pH at approximately 6.0.

#### HARVESTING AND SAP PREPARATION METHODS

At harvest the plants were separated into roots and tops by cutting the stalk just above the whorl of adventitious roots. The roots were washed twice in distilled water and the excess removed by whirling the roots in a cheesecloth bag under comparable conditions for each set. Fresh weights were taken on tops and roots, the material cut into one-inch lengths, thoroughly mixed and subsamples taken for moisture determinations by drying for 48 hours in a 70° C. oven. This dried material was ground finely in a Wiley mill and used for sugar, total nitrogen, and "true protein" determinations. The remainder of the fresh material was wrapped in cheese cloth, placed in individual glass jars with screw caps and quickly frozen in the freezing compartment of a refrigerator. Until analyses could be made the frozen material was stored at -20° F. The commonly determined nitrogen fractions are not altered by freezing and frozen storage (21).

Investigations (1, 30) have shown that successive increments of expressed sap from plant tissues previously frozen are similar in composition with respect to ions and compounds in true solution and that estimates of composition can be calculated to a dry weight basis as accurately from sap analyses as from analyses on dried material (30). Therefore, sap analyses was chosen since glutamine is unstable unless the green material can be dried rapidly under carefully controlled conditions (27, 42). Soluble nitrogen fractions estimated on the sap are calculated to a dry weight basis on the assumption that the sap remaining in the press cake, estimated from its moisture content, is similar in composition to that expressed. Corrections have not been made for total solids in the sap, and the values calculated in this way may be slightly low (30). The frozen material was thawed at room temperature and the sap expressed from the tissue wrapped in cheesecloth, at 2600 lbs./sq. in. in a hydraulic press. The sap was yellow to brown in color and contained considerable colloidal material. It was stored in the refrigerator under toluene until analyses were completed.

#### ANALYTICAL METHODS

**DRY MATERIAL.**—Reducing sugars and sucrose were determined on 80 per cent. alcohol extracts of the ground dry tissue according to the procedure of HASSID (6), sucrose being calculated as the difference in reducing value of extracts before and after inversion with 1 N  $\text{H}_2\text{SO}_4$  at room temperature for 24 hours. The soluble sugars of corn are principally glucose, fructose,



and sucrose (17). Total nitrogen and "true protein" nitrogen were determined by the Kjeldahl-Gunning method, the latter on the residue from the alcoholic extraction for sugars followed by exhaustive extraction with boiling water by a procedure similar to that recommended by VICKERY *et al.* (44). Total nitrogen determinations on dry material and sap were not modified to include nitrate since the nitrogen depletion of the plants prior to experiment reduced this constituent to a low level.

SAP.—Ammonia nitrogen was run, the same day the sap was expressed, by the procedure of PUCHER, VICKERY, and LEAVENWORTH (27). Glutamine and asparagine amide nitrogen were determined the following day by the differential-hydrolysis method of VICKERY, PUCHER, *et al.* (42) on the sap preserved overnight at 2° C. Apparently amide nitrogen pre-existing in proteins and protein degradation products, which are present in the sap, is not obtained in the amide fractions since KEYSSNER and TAUBOCK (14) have shown that no amide nitrogen could be obtained from l-leucyl glutamine until after the peptide linkage was hydrolyzed. Peptide hydrolysis is probably negligible under the conditions of hydrolysis used for the estimation of asparagine amide nitrogen. Urea interferes in the determination of the amides (42), but it has been shown to be absent in corn (16). That it is at least negligible has been confirmed by 4-hour hydrolysis as compared with 2-hour hydrolysis of aliquots of sap in pH 6.5 buffer followed by distillation of the ammonia. Identical results indicated the absence of urea and allantoin (42). Urease hydrolysis followed by ammonia distillation failed to indicate the presence of determinable amounts of urea in the sap. Sap pH was determined with a glass electrode and potentiometer circuit.

Total soluble,  $\alpha$ -amino and peptide nitrogen were determined on sap which had been deproteinized with 3 gm. trichloroacetic acid per 100 ml. of sap (pH approximately 0.8) followed by the removal of the coagulum by filtration through quantitative filter paper. Trichloroacetic acid does not precipitate all of the degradation products of proteins (i.e., peptones, polypeptides, etc.) from solution (5, 9, 22). This is true of corn as shown by evidence presented later. Total soluble nitrogen was determined by the Kjeldahl-Gunning method.  $\alpha$ -Amino nitrogen was determined in a Van Slyke apparatus equipped with a microburette. Since ammonia and glutamine acid nitrogen interfere in the Van Slyke procedure for  $\alpha$ -amino nitrogen (4, 42), they were removed by hydrolyzing aliquots of deproteinized sap for 2 hours with 2 N HCl at 100° C. and distilling the ammonia on a steam bath from the solution made slightly alkaline to bromocresol purple.<sup>2</sup> The solution was then acidified with acetic acid and diluted to volume for the determination. However, the hydrolytic procedure leads to the formation of some humin, and, hence, the values reported herein are undoubtedly low. From the total  $\alpha$ -amino nitrogen the  $\alpha$ -amino nitrogen of glutamine and asparagine equivalent to the amide nitrogen is subtracted and the result expressed for convenience as residual  $\alpha$ -amino nitrogen in common with the

<sup>2</sup> Personal communication from T. C. BROYER *et al.*, University of California.

practice of Wood and co-workers (47, 48, 49). Other procedures used will be discussed in connection with the results.

### Experimental results

The growth and analytical results for the roots and tops of plants of experiments 1 and 2 for the period of ammonium absorption are shown in tables I and II respectively. The nitrogen fractions determined on the expressed sap have been calculated to a dry weight basis for reasons that will be apparent below. The  $(\text{NH}_4)_2\text{SO}_4$  concentrations indicated are greater than the actual amount in the solution because of absorption, which although large, is small in relation to the ammonium available.

#### GROWTH AND GENERAL APPEARANCE

The objective in experiment 1 was to gradually increase the ammonium concentration of the solution until the plants were definitely injured. At the end of 6 days the leaves were showing a slight dieback at the ends and tiny yellow blotches on the blades, but pronounced injury was not apparent until the end of 13 days when the plants had been in 120 meq./l.  $(\text{NH}_4)_2\text{SO}_4$  for 7 days. The leaf tips showed considerable injury and large necrotic areas appeared on the blades. The leaves were rolled, indicating a severe water deficit, and the roots were turning brown. The roots, however, were apparently still living as indicated by their ability to exclude ammonium, which had a concentration of 1,450 mg.  $\text{NH}_3\text{-N/l.}$  by analysis in the final culture solution as compared with 140 mg.  $\text{NH}_3\text{-N/l.}$  in the expressed sap. In experiment 2 in which the ammonia concentration of the external solution was not raised to as high a level, a few of the leaf blades were showing white necrotic areas at the end of 7 days, and during the day when the greenhouse temperature was 80–90° C. the leaves showed signs of severe moisture deficit.

The data show that during the period of ammonium nutrition the plants gained appreciably in dry weight in both the tops and in the roots. The most conspicuous effect of ammonium nutrition of corn under these conditions is the decrease in moisture content of the tops of the plants, and to a lesser extent in the roots, evidencing itself in the loss of turgor and the in-rolling of the leaves.<sup>3</sup> Because of this loss of water from the tissues, the changes in soluble nitrogen compounds on a dry weight basis are not as great as they are on a sap concentration basis. Thus the factor to convert mg. N/l. of sap to mg. N/kg. dry weight decreases from 11.25 for the control (0 days) to 7.06 for the tops of plants harvested at 13 days in experiment 1. However, the magnitude of the increases in various nitrogen compounds is so great

<sup>3</sup> Since this manuscript was submitted, G. J. RALEIGH (Science n. s. 103: 206–207. 1946) has shown that limiting the supply of nitrogen to produce low-nitrogen and presumably high-carbohydrate rye grass plants is essential to obtain the exudation of glutamine when  $\text{NH}_4\text{Cl}$  is supplied to plants in soil. In the experiments reported here,  $(\text{NH}_4)_2\text{SO}_4$  supplied in nutrient solution to low-nitrogen, high-carbohydrate corn plants failed to produce guttation. Whether this is due to an immediate effect of ammonium nitrogen on the absorption of water by corn roots thus preventing guttation is not known. However, the effect of ammonium nitrogen in decreasing the water content of corn tops after 3 days is shown in table I and may lend support to this hypothesis.

that the same general picture is obtained whether the data are compared on a sap concentration (45) or on a dry weight basis.

#### CHANGES IN CHEMICAL COMPOSITION

The data for tops and roots in both experiments show that the tissues increased in total nitrogen and that the increase was largely confined to an increase in the total soluble nitrogen with the possible exception of the second experiment in which there appeared to be a significant increase in the "true protein" nitrogen of the tops. This accumulation of total soluble nitrogen was so great that in experiment 1 the soluble nitrogen amounted to over 60 per cent. of the total nitrogen of the tops at the end of the experiment as compared with 25.7 per cent. for the tops after growth in the  $(\text{NH}_4)_2\text{SO}_4$  containing solution for 3 days. Large increases in glutamine, asparagine, and residual amino nitrogen occurred in the roots and tops in both experiments. A slight but probably significant upward shift in the pH of the sap occurred during the period of ammonium nutrition, perhaps due to the neutralization of organic acids by the gradual accumulation of ammonium, but the pH is much higher than in those "acid plants" which can accumulate large amounts of ammonium as the cation of organic acids (28, 29). The pH of the sap did not become alkaline as MEVIUS (19) found for corn grown in ammonium solutions of alkaline reaction. In no case did ammonium accumulate before the other soluble nitrogen constituents had reached relatively high levels and the plants were showing or beginning to show evidence of injury.

The level of carbohydrates, as measured by total sugars or reducing sugars and sucrose, remained comparatively high throughout the experimental period indicating that available carbohydrate at no period during ammonium nutrition could have been limiting the assimilation of ammonium. In both experiments there appears to be a significant downward trend in the total sugars of the roots, which may be related to the site of ammonium assimilation to be discussed later.

In experiment 1 (table I) the "non-amino basic nitrogen" determined on the tops also shows a large increase. This fraction was obtained by subtracting from the nitrogen precipitated by phosphotungstic acid from the trichloroacetic acid filtrates, according to the procedure of UMBREIT and WILSON (35), the difference in amino nitrogen of the trichloroacetic acid filtrate and the filtrate after the precipitation of "basic nitrogen." As previously pointed out the trichloroacetic acid filtrate contains appreciable amounts of polypeptides and higher polymers of amino acids; and hence included in the "non-amino basic nitrogen" is much peptide nitrogen (35, 22), the non- $\alpha$ -amino portion of basic amino acids, cyclic nitrogen compounds, and unknown entities containing nitrogen. In spite of the indefinite character of this material the data do indicate that the accumulation of compounds other than ammonia, glutamine, asparagine and residual amino nitrogen has occurred. Subtraction of the sum of all of these mentioned categories of nitro-

gen from the total soluble nitrogen for the tops reveals that there is still considerable "rest" or unidentified nitrogen in the sap and that this fraction also increases during ammonium nutrition.

To get further information on the nature of the compounds elaborated a more detailed study was made of the sap of the tops of plants in experiment 2 (table II). Aliquots of sap deproteinized with 3 per cent. trichloroacetic acid were acid-hydrolyzed and  $\alpha$ -amino, humin, phosphotungstic-acid

TABLE I

CHEMICAL COMPOSITION, GREEN AND DRY WEIGHTS, AND MOISTURE CONTENT OF THE TOPS AND ROOTS OF 12 CORN PLANTS GROWN IN A LOW-NITROGEN SOLUTION AND THEN TRANSFERRED TO A COMPLETE NUTRIENT SOLUTION CONTAINING  $(\text{NH}_4)_2\text{SO}_4$  FOR THE NUMBER OF DAYS INDICATED. THE  $(\text{NH}_4)_2\text{SO}_4$  CONCENTRATIONS ARE THOSE OF THE CULTURE SOLUTION FOR THE INTERVAL SINCE THE PRECEDING HARVEST

Days	0	3	6	13
$(\text{NH}_4)_2\text{SO}_4$ , meq./l.	0	30	60	120
TOPS				
Green wt., gm.	342*	295	373	357
Dry wt., gm.	27.4*	27.7	38.8	44.5
% moisture	92.0	90.6	89.6	87.5
pH of sap	6.65	6.66	6.90	6.88
Reducing sugar, % dry wt.	2.95	4.37	3.55	3.78
Sucrose (as glucose), "	2.05	1.75	2.91	1.84
Total N, mg. N/kg. dry wt.	26,500	28,400	30,300	34,800
Ammonia, "	172	127	166	706
Glutamine, "	307	381	870	2,320
Asparagine, "	420	930	2,190	4,150
Residual $\alpha$ -amino, "	1,750	1,400	1,760	2,680
Non-amino basic, "		755	2,200	2,620
Undetermined sol., "		3,732	5,814	8,624
Total soluble, "		7,325	13,000	21,100
"True protein," "	18,800	21,200	18,800	18,800
ROOTS				
Green wt., gm.	87.0*	86.3	86.0	84.5
Dry wt., gm.	5.47*	5.94	5.58	6.08
% moisture	93.7	93.1	93.5	92.8
pH of sap	6.70	6.73	6.95	6.96
Total sugar, % dry wt.	5.72	5.63	4.37	4.82
Total N, mg. N/kg. dry wt.	14,950	21,200	28,400	32,600
Ammonia, "	415	553	905	1,820
Glutamine, "	425	3,180	2,100	3,560
Asparagine, "	554	2,720	3,080	7,340
Residual $\alpha$ -amino, "	823	3,110	3,920	6,020

\* Calculated from the weights for 6 plants.

precipitable, and alkaloid nitrogen determined on the hydrolysate. Fifty-ml. aliquots of trichloroacetic acid treated sap were refluxed for 16 hours with 0.2 gm. stannous chloride and 50 ml. concentrated HCl. In common with the experience of others on plant sap (22), the presence of a reducing agent did not prevent the formation of humin. The humin was filtered off and the filtrate concentrated on the steam bath to drive off the HCl. It was then made alkaline to bromocresol purple and heated on the steam bath to drive off any ammonia liberated during hydrolysis. The solution was filtered, acidified with acetic acid, and  $\alpha$ -amino nitrogen run on the filtrate. The

difference between  $\alpha$ -amino nitrogen before and after hydrolysis is reported in table II as peptide nitrogen although admittedly a portion of the tryptophane, cystine and tyrosine and possibly other amino acids were probably lost. [See the review of Lugg (18).] Nevertheless, the data do indicate that there was a considerable amount of peptide and similar nitrogen in the sap not precipitated by trichloroacetic acid under the conditions employed; and that this nitrogen increases more than 8-fold during ammonium nutrition.

The humin nitrogen resulting from the filtration of the hydrolysate and that obtained by filtration after concentration and neutralization of the hydrolysate were combined and total nitrogen determined. Table II indicates that there was also a significant increase in this fraction during ammo-

TABLE II

CHEMICAL COMPOSITION, GREEN AND DRY WEIGHTS, AND MOISTURE CONTENT OF THE TOPS AND ROOTS OF 15 CORN PLANTS GROWN IN A LOW-NITROGEN SOLUTION AND THEN TRANSFERRED TO A COMPLETE NUTRIENT SOLUTION CONTAINING 60 MEQ./L.  $(\text{NH}_4)_2\text{SO}_4$  FOR 7 DAYS

	Tops		Roots	
	0	7	0	7
Days in solution .....	0	7	0	7
Green wt., gm. ....	426	375	153	141
Dry wt., gm. ....	50.7	56.9	11.8	14.1
% moisture ....	88.1	84.8	92.3	90.0
pH of sap .....	5.38	5.40	5.24	5.24
Total sugar, % dry wt. ....	7.95	9.50	5.84	4.05
Nitrogen fractions:				
Total, mg. N/kg. dry wt. ....	17,200	23,000	10,800	25,500
Ammonia, " .....	43.9	225	232	663
Glutamine, " .....	93.8	897	146	1,310
Asparagine, " .....	205	1,720	225	1,940
Residual $\alpha$ -amino, " .....	730	2,470	1,480	1,860
Peptide, " .....	173	1,440	.....	.....
Humin, " .....	360	422	.....	.....
Undeter. sol., " .....	744	726	.....	.....
Total soluble, " .....	2,350	7,900	2,910	8,600
"True protein," " .....	11,700	12,700	8,000	12,000
Basic, " .....	100	192	.....	.....

nium nutrition. This is not surprising in view of the probable source of humin from amino acids condensing with aldehydes, etc., and to the fact that the peptides and amino nitrogen had increased during the same interval.

Since the humin nitrogen probably came from either the free amino nitrogen or from the amino nitrogen resulting from polypeptide hydrolysis (thereby making the apparent peptide nitrogen less than its probable value since it is obtained by difference) it can be legitimately added to the determined forms of nitrogen in table II of the sap of the tops to calculate the undetermined or "rest" nitrogen by subtraction from the total soluble nitrogen. The data indicate no significant change in this "rest" nitrogen.

In view of the evidence that the leaf proteins of corn contain over 14 per cent. arginine (3), it was desired to determine to what extent this amino acid was a portion of the amino acids synthesized. Because of the lack of material for precipitation and purification of arginine, the basic amino acids were



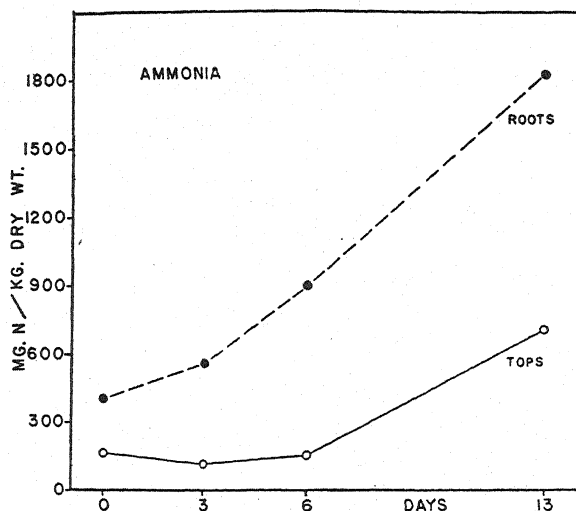


FIG. 1. Ammonia nitrogen did not increase in the tops of corn until after injury symptoms appeared and ammonia nitrogen had accumulated in the roots.

precipitated from the hydrolysate mentioned above by the procedure of UMBREIT and WILSON (35) except that the precipitating volume was doubled by the addition of distilled water and enough sulphuric acid to maintain the sulphuric acid at the concentration recommended. Dilution is necessary because of the low solubility of the phosphotungstates of some of the non-basic amino acids according to Vickery in a communication to THOMAS (34). Table II indicates that the nitrogen precipitated by this reagent during the period of ammonium nutrition is almost doubled. The increase of 92 mg. N/kg., in the basic nitrogen, only a small portion of which is probably  $\alpha$ -amino nitrogen determinable by the Van Slyke procedure, is only a small fraction of the increase of 1740 mg. residual  $\alpha$ -amino N/kg. noted. There-

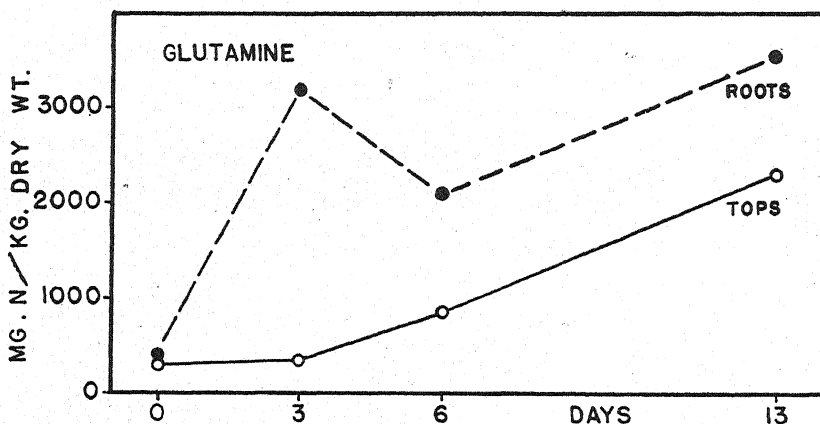


FIG. 2. Glutamine nitrogen increased more rapidly in the roots than in the tops of corn supplied with ammonium nitrogen. The decrease of glutamine nitrogen in the roots between 3 and 6 days is discussed in the text.



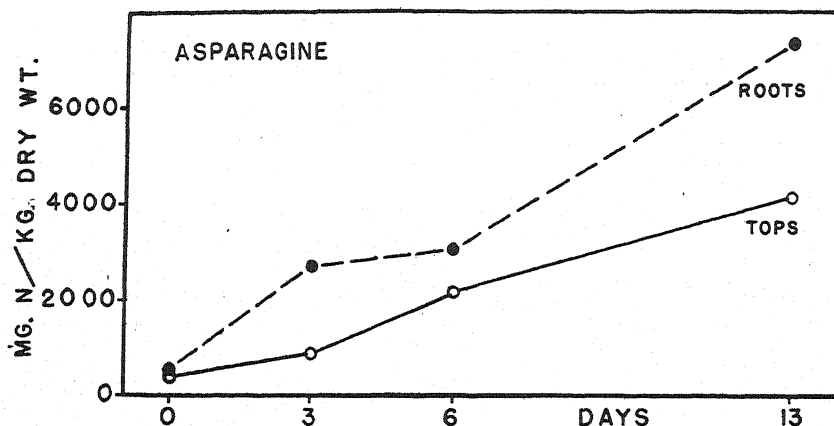


FIG. 3. Asparagine nitrogen increased more rapidly in the roots than in the tops of corn supplied with ammonium nitrogen.

fore, it appears probable that histidine, lysine, and arginine do not constitute an important portion of the residual amino nitrogen elaborated.

As previously mentioned, alkaloids are generally considered to be very low or non-existent in the Monocotyledoneae, but it was deemed advisable to determine if there was any synthesis of this class of compounds under the special conditions imposed by the experiments. Alkaloid nitrogen was estimated on the hydrolysate of experiment 2 by the colorimetric determination of bismuth precipitated by the combination of alkaloids and betaines with  $\text{KBiI}_4$  according to the procedure of KLEIN and LINER (15). Using their atomic ratio Bi:N equals 1:2 for the precipitate as determined for stachydrine and trigonelline, this form of nitrogen was found to amount to about 10 mg./kg., an insignificant proportion of the soluble nitrogen, and showed little variation under the conditions of ammonium nutrition.

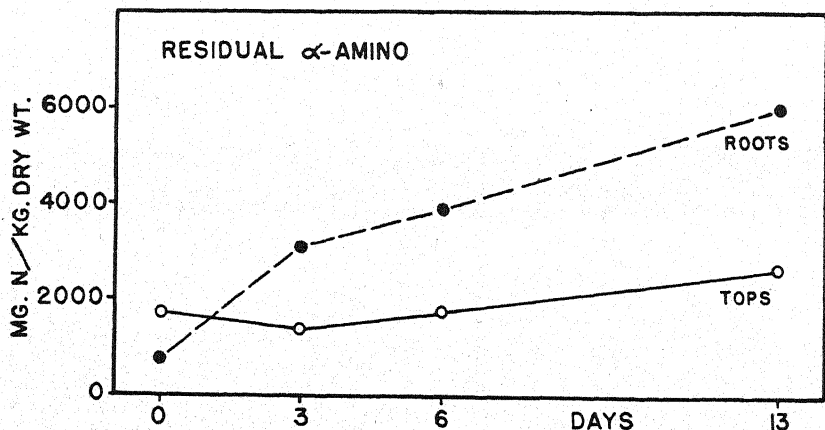


FIG. 4. Residual  $\alpha$ -amino nitrogen, like glutamine and asparagine nitrogen, accumulated more rapidly in the roots than in the tops of corn.

## THE SITE OF AMMONIUM METABOLISM

The data of table I graphed in figures 1, 2, 3, and 4 for ammonia, glutamine, asparagine, and residual amino nitrogen, respectively, on a dry weight basis for the roots and tops show the rate of accumulation of these compounds. From figure 1 it is apparent that the ammonium gradually increases in the roots as the external concentration was raised, but that in the tops it did not accumulate until it had reached a relatively high level in the roots. Glutamine, asparagine and residual amino nitrogen accumulated more rapidly and reached much higher values in the roots than they did in the tops.

In figure 2 a loss of glutamine is indicated from the roots for the interval 3 to 6 days which amounted to a total of 7.1 mg. of glutamine nitrogen, but the tops gained during the same period 19.7 mg. of glutamine nitrogen, evidently translocated from the roots. That there may be rapid translocation from root to shoot of amide nitrogen has been shown in experiments to be reported later in which plants purposely increased in amide content by the technique described here and then transferred to solutions lacking nitrogen rapidly lost practically all of the glutamine and part of the asparagine from the roots.

As in all experiments with intact plants relative amounts of constituents may not reflect the primary centers of synthesis because of rapid translocation. However, the evidence offered here suggests that the ammonia is largely combined with organic precursors of the amides and amino acids in the roots and that these are translocated upward into the tops. Consistent with the explanation that ammonia is largely metabolized in the roots is the fact that ammonia does not appear in the tops in appreciable amounts until the roots have apparently reached their capacity to assimilate it as fast as it is absorbed. This formation of amides and amino acids in the roots has its parallel in other work since excised roots of barley in ammonium solutions elaborate amides, largely glutamine, which accumulate in the roots (10), beet roots synthesize glutamine from absorbed ammonium (40), and apple rootlets reduce nitrate with the formation of amino acids (33).

## Discussion

One of the most conspicuous effects of ammonium nutrition on corn was the rolling of the leaves and other indications of moisture deficit within the plant. This was apparent in the decrease in moisture content of the tops before the leaves showed visible indications. A similar effect of ammonium salts has been noted for the grasses, *Phalaris tuberosa* L. and *Lolium multiflorum* Lam. (25), and ammonium as compared with nitrate nutrition of cotton (12). VICKERY *et al.* (44), however, found that tobacco growing in sand culture with varying proportions of ammonium to nitrate nitrogen were higher in moisture at the higher ammonium levels. HAYWARD and SPURR (7) have shown that increasing concentrations of salts markedly lower the water absorption of corn roots and attributed this effect to the

increasing osmotic concentrations. Although the relatively high osmotic pressure of the solutions containing  $(\text{NH}_4)_2\text{SO}_4$  may have contributed to the low moisture content of the tops of the plants in the experiments reported in this paper, when similar plants were transferred to solutions lacking  $(\text{NH}_4)_2\text{SO}_4$ , in experiments to be reported later, the plants continued to show symptoms of severe water deficit to the termination of the experiment (8 days) indicating that the water deficit was dependent to a great extent on changes occurring during the period of ammonium nutrition. PEARSALL and EWING (23) concluded from experiments with a variety of plants grown with different levels of nitrate in sand culture that the increased water content noted with increasing nitrogen supplies was dependent on the increased pH of the expressed sap affecting the swelling of the proteins. However, in the experiments reported here decreasing water content was noted with increasing supplies of ammonium sulphate and with increasing pH of the expressed sap. It is evident that the relations between water content, pH, and nitrogen nutrition are complex. Whether the loss of turgor noted in these experiments was due to depressed absorption of water, to a higher rate of transpiration, or was due to some physical or metabolic factor or factors controlling the hydration of colloidal and cell surfaces was not determined.

Tables I and II indicate that there has been little change in the level of "true protein" even though the quantities of total soluble nitrogen, amides, amino acids, and other forms of soluble nitrogen increased greatly during the period of ammonium nutrition. Because attempts at isolating and purifying the proteins of vegetative tissue have led to very poor yields (2, 38), the investigator for the present at least, must be content with using some arbitrarily chosen solvent or group of solvents under varying conditions of concentration, pH, and temperature in determining what is protein and what is not protein. That class of compounds lying intermediate between the amino acids and the true proteins and even some of the free amino acids themselves may be soluble or insoluble depending upon the "protein" precipitant and the exact conditions of its use. With these precautions in mind when "true protein" is considered it is apparent that the experimental conditions altered the protein level but little in spite of the injury and the increases in amino nitrogen obtained. Therefore, the net increases in amides, amino acids and other soluble compounds must have come, for the most part, from the ammonium absorbed rather than from the breakdown of proteins. This assumption, of course, does not ignore that there may not have been an exchange of nitrogen from the soluble forms for the nitrogen in the proteins in a protein cycle as has been demonstrated with tobacco (43) and with sunflowers (8) by isotopic technique or that a part of the compounds synthesized went into the formation of new protein incident to growth. Hence, these experiments in many respects are not comparable with those on germinating seeds, detached leaves growing in the dark or in the light, or with plants in the dark in which there is a net decrease in pro-

tein and the amino acids and even the amides can come either from the hydrolysis of the protein (primary origin) or from secondary reactions following the deamination of a part of the amino acids (secondary origin).

CHIBNALL (2) summarizes the many theories of protein regulation in the plant that have been advanced among which are the amino acid level, the oxygen supply, the level of carbohydrate and ammonia compounds acting through the "mass-action law," the respiratory rate and the water content theories, but the more modern concepts regard protein as being maintained at a super-optimum level by a continuous expenditure of energy (24, 26). The results given in this paper with corn show that in spite of the decreasing water content and increasing amino nitrogen content resulting from ammonium nutrition and with an abundance of carbohydrate present, there was little effect on the protein content. In corn, evidently, factors more decisive than water content, amino acid level, or potential ammonia compounds and abundant carbohydrate regulate the protein level.

TABLE III

INCREASE IN SOLUBLE NITROGEN CONSTITUENTS EXPRESSED IN MG. N/KG., PERCENTAGE OF THE INCREASE IN TOTAL SOLUBLE NITROGEN, AND IN PERCENTAGE OF THEIR ORIGINAL VALUES FOR THE ROOTS AND TOPS OF CORN PLANTS AS CALCULATED FROM TABLE II

	TOPS			ROOTS		
	mg./kg.	% TSN*	% of orig.	mg./kg.	% TSN*	% of orig.
Total sol. N (TSN) .....	5550	.....	237	5690	.....	195
Ammonia N .....	181	3.3	413	431	7.6	186
Glutamine N .....	803	14.5	860	1164	20.5	798
Asparagine N .....	1515	27.3	738	1715	30.2	763
Res. $\alpha$ -amino N .....	1740	31.4	239	380	6.7	26
Peptide N .....	1267	22.8	732	.....	.....	.....
Humin N .....	62	1.1	17	.....	.....	.....
Basic N .....	92	1.7	92	.....	.....	.....

\* TSN = Total soluble nitrogen.

The increase in soluble nitrogen and other nitrogen fractions for the tops and roots of the plants of experiment 2 in mg. N/kg. and in percentage of the increase in soluble nitrogen and in percentage of the original values is shown in table III. The apparent amounts of various compounds synthesized and the percentage of each synthesized in relation to the increase in total soluble nitrogen and in percentage of the original values is shown in table IV. The asparagine synthesized on a nitrogen basis is approximately twice the amount of glutamine produced. This was also apparent for the first experiment in which 12 plants for a 13-day period synthesized 214 mg. asparagine nitrogen and 114 mg. of glutamine nitrogen or 1.89 times as much asparagine as glutamine. This does not mean, however, that analysis of corn will always show twice as much asparagine as glutamine. In this laboratory, analysis of samples of relatively mature stalks and leaves grown under field conditions have frequently shown more glutamine than asparagine. This

may be due as SCHWAB (31) has shown to the fact that the typical amide of a plant is both synthesized and decomposed more rapidly than the other. Therefore, under certain conditions in which amides are being depleted in corn, glutamine might occur in excess of asparagine.

In table III the data for tops and in table IV the data for the entire plant indicate that residual amino nitrogen may occupy a position as important as that of asparagine in the metabolism of ammonium. However, this is not always true as indicated by experiment 1 in which the gain in total residual  $\alpha$ -amino nitrogen amounted to less than half the increases in total asparagine nitrogen for 12 plants for the periods 0-3 days, 0-6, and 0-13 days. Table I indicates that all of this gain of residual amino nitrogen for the 0-3- and 0-6-day periods was in the roots rather than in the tops. The limited data of table III for peptide nitrogen indicate that this fraction also accounts for a large part of the increase in soluble nitrogen and that the basic nitrogen and humin nitrogen account for but little of the increase.

TABLE IV

INCREASE IN CERTAIN SOLUBLE NITROGEN CONSTITUENTS OF 15 CORN PLANTS EXPRESSED AS MG. NITROGEN, AS PERCENTAGE OF THE INCREASE OF TOTAL SOLUBLE NITROGEN, AND AS PERCENTAGE OF THE ORIGINAL VALUES FOR EACH CONSTITUENT AS CALCULATED FROM TABLE II

	<i>mg. N</i>	<i>% TSN</i>	<i>% of original</i>
Total soluble N (TSN) .....	416	.....	272
Ammonia N .....	17.3	4.2	353
Glutamine N .....	62.8	15.0	968
Asparagine N .....	112	26.9	862
Res. $\alpha$ -amino N .....	112	26.9	205

The data of tables III and IV indicate that the various categories of nitrogen compounds do not increase in equal proportion when ammonium is supplied. Thus for 15 plants the increase in glutamine was 9.68 times the original value whereas the increase of residual amino nitrogen amounted to only 2.05 times its original value. Hence, no simple equilibrium relations exist between the ammonia, the respective amides, and the other compounds, but that the proportions are controlled by the enzyme systems and the energetics of the protoplasm itself.

These experiments place corn in that group of plants which contain appreciable amounts of both glutamine and asparagine and are capable of synthesizing both amides under appropriate conditions as distinct from the group which accumulate only glutamine like the beet (40) and the tomato (41) or that group which accumulates only asparagine like Sudan grass and Kikuyu grass (47, 48). CHIBNALL (2) and VICKERY and PUCHER (39) have considered amide formation to be a regular phase in the respiratory metabolism of the plant in contrast with the older view that their occurrence was for the sole purpose of detoxicating ammonia. In accordance with this view the accumulation of ammonia eventually noted in the sap of the plants in



these experiments can be regarded as a consequence of a rate of absorption exceeding the capacity of the system to metabolize it to amides, amino acids, and other nitrogen compounds perhaps due to the limited rate at which certain essential precursors are produced.

Several investigations (23, 25, 32) have shown that the amino acid content of a plant may be increased either by the amount or the form of nitrogen supplied. Unfortunately, in many investigations ammonia and glutamine amide nitrogen are not removed before the  $\alpha$ -amino nitrogen is determined; so that the resulting values are higher than they should be. If this correction is made and if the treatments are compared on a residual-amino nitrogen basis, there is frequently little change. These experiments show that the residual amino nitrogen may be altered by ammonium nutrition, and hence, this fraction in corn occupies an important position in the assimilation of ammonium. Evidence has shown that only a small part of the increase observed can be in the basic amino acid fraction. The remainder of the increase may consist of a whole series of amino acids, or it may be an increase in only one amino acid. There is the possibility that glutamic or aspartic acids may be synthesized in addition to that proportion of each associated with the respective amides. However, WOOD and CRUICKSHANK (47) have shown with Kikuyu grass that the aspartic acid content closely paralleled that of the asparagine. That the amino acids are not synthesized in the same proportion as they occur in the leaf proteins has been indicated by the low proportion of basic amino nitrogen synthesized in relation to its proportion in the proteins of corn and by the large amounts of glutamic acid and aspartic acid associated with the amides. WOOD and CRUICKSHANK (47) have shown that in starving grass leaves in which proteins were being hydrolyzed, the amino acids are preferentially oxidized, cystine being the most rapid; and this results in a different proportion in the free amino acids as compared with those in the original protein. In these experiments with corn, there is no reason to believe that the amino acids would be synthesized in proportions similar to that of the proteins. If such a phenomena did occur differential oxidations might alter the proportions.

### Summary

1. Corn plants previously depleted in soluble nitrogen compounds and showing nitrogen deficiency symptoms on the lower leaves rapidly absorb nitrogen from a complete nutrient solution containing  $(\text{NH}_4)_2\text{SO}_4$  and continue to increase in weight.
2. In time studies in which the  $(\text{NH}_4)_2\text{SO}_4$  concentration was gradually increased to produce toxicity symptoms, injury did not appear until the soluble nitrogen constituents of the plant sap had reached relatively high levels. The most notable toxicity symptoms were severe water deficit and necrotic areas on the leaves and leaf tips.
3. Analysis of the expressed sap showed that large amounts of glutamine, asparagine,  $\alpha$ -amino and other forms of soluble organic nitrogen accumu-



lated in the roots and tops. Ammonium did not increase rapidly until the soluble nitrogen had reached high values. There was a slight increase in pH of the sap of roots and tops. There was little change in total sugar or "true protein" determined on dry material.

4. Sap analysis on plants in a similar experiment indicated that part of the increase in soluble nitrogen was due to peptides and compounds that formed humin on acid hydrolysis of the deproteinized sap. Only a very small portion of the  $\alpha$ -amino nitrogen synthesized is basic amino acids. Undetermined soluble nitrogen and alkaloid nitrogen remained constant during ammonium nutrition.

5. Comparative rates of accumulation of nitrogen compounds in the roots and tops suggest that absorbed ammonium is metabolized to asparagine, glutamine, and  $\alpha$ -amino nitrogen mainly in the roots and subsequently translocated to the shoots.

6. On a nitrogen basis asparagine accumulates twice as rapidly as glutamine. One or more amino acids, other than glutamic and aspartic associated with the amides, may be quantitatively as important as asparagine in the metabolism of ammonium by corn. The accumulation of  $\alpha$ -amino nitrogen in the apparent absence of net protein hydrolysis is discussed.

7. The "true protein" level is discussed in relation to water, amino acid, and sugar contents and to current theories of protein regulation.

EXPERIMENT STATION CHEMISTRY DEPARTMENT

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## THE EFFECT OF COPPER DEFICIENCY ON THE NITROGEN METABOLISM AND OIL SYNTHESIS OF THE TUNG TREE

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A recent review (6) indicates that most of the literature on copper deficiency in plants is concerned with its occurrence and correction under field conditions. Only a few studies are cited as evidence of essentiality of copper in nutrient culture because of the experimental difficulties involved. This element, however, is now regarded as essential to plant growth, though its specific physiological rôle has not been determined. Recently BAILEY and McHARGUE (5), comparing the enzymatic activities of copper-deficient and normal tomato plants, found that the only exceptional enzyme activity that could be correlated with increasing increments of copper was that of pyrogallal oxidase. ARNON (1) has found that copper is beneficial in promoting the growth of barley in non-aerated solutions, but his data do not show the definite rôle of copper in the metabolism of the plant. An interesting suggestion has been offered by CAMP and FUDGE (8) that in citrus the relationship between nitrogen and copper may be so important that the symptoms may more properly be regarded as a consequence of an unbalanced copper-nitrogen ratio than of copper deficiency alone.

Recently, a disorder of tung trees (*Aleurites fordii* Hemsl.) has been described by DROSDOFF and DICKEY (11). They corrected the disorder by the application of copper sulphate to the soil around the tree or to the leaves as a spray. They found that in July normal trees had, on a dry-weight basis, 5 or more p.p.m. of copper in the midshoot leaves, whereas similarly located leaves on severely affected trees had only 2 to 3 p.p.m. of copper. The occurrence of this disorder in a commercial orchard of bearing 8-year-old trees near Morriston, Florida, provided material for a study of its effect on the seasonal content of reserve food material in the leaves and the synthesis of oil and other reserves in the fruit.

### Methods

In the early summer of 1942, the trees were rated as to the extent of the injury to the foliage caused by the deficiency. Four plots of 10 trees each were selected, 2 of the plots having trees uniformly affected with the trouble and, in an adjacent area of the same orchard, 2 plots having normal appearing trees. A composite sample of 20 midshoot leaves from each tree or 200 total was taken to represent each plot. Care was taken to avoid the severely necrotic terminal leaves of the deficient trees, the samples being representative of the midshoot leaves exhibiting incipient deficiency symptoms. Composite samples of 30 fruits, 3 from each of the 10 trees, were also taken at random from positions representative of the bearing surface of each tree. Both leaf and fruit samples were obtained on June 28, August 15, and Octo-



ber 23, to correspond with the respective periods: (I) just preceding synthesis of oil in the kernels, (II) mid-point of active oil formation, and (III) maturation of the kernel, as had been indicated by previous studies (21).

The composite samples of leaves and fruit were separately prepared for analysis essentially as described by SELL *et al.* (21). Aliquots of each composite were preserved in 80 per cent. ethanol for carbohydrate analyses. Separate aliquots for the determination of dry weight were taken, and these samples were heated to 105° C. for 5 minutes, then dried for at least 12 hours at 70° C. in an oven with forced draft. The kernel fraction of the fruit was flaked on an inverted carpenter plane and dried to constant weight over phosphorus pentoxide in a vacuum oven at 70° C. and 4-mm. pressure. After drying, the samples, other than kernel samples for oil determinations, were ground in an Intermediate Wiley mill, using a 60-mesh screen. This material was used for the determination of total and water-soluble nitrogen and copper. Grinding in this mill had been found in preliminary trials to give no detectable contamination with copper, as has also been reported by BAILEY and MCHARGUE (4).

The carbohydrate fractions were determined exactly as described by SELL *et al.* (21). Total nitrogen was determined by the Kjeldahl procedure, A.O.A.C. (2). Water-soluble nitrogen was determined by digesting a 10-gm. sample of the dried material with 250 ml. of distilled water in a covered 400-ml. beaker for one-half hour on a steam bath. The contents of the beaker were then filtered through a 9-cm. #00 Munktell filter paper on a Buchner funnel into a suction flask. The residue on the filter paper was then washed with four 50-ml. portions of hot distilled water, the separate portions being added from a graduated cylinder to avoid channeling of the residue during washing. The filtrate was then transferred to a 500-ml. volumetric flask and made to volume with the rinsings of the suction flask. A 200-ml. aliquot was transferred to an 800-ml. Kjeldahl flask, 1 ml. of concentrated sulphuric acid added and the water removed by evacuation while on a steam bath. The nitrogen of the aliquot was then determined by the standard Kjeldahl method, using salicylic acid in the digestion mixture, although colorimetric tests had failed to indicate the presence of nitrate in any of the samples of leaves and fruit. Biuret tests for polypeptides on the water-soluble fractions proved negative.

Another 200-ml. aliquot was transferred to a 300-ml. standard taper Erlenmeyer flask, 20 ml. of 12 N  $\text{H}_2\text{SO}_4$  added, and the solution was refluxed in a boiling water bath for 3 hours (20). The digest was then transferred to a Kjeldahl flask, washing five times with 60-ml. portions of water, 35 ml. of 10 N NaOH added, and the ammonia distilled. The distillate was collected in an Erlenmeyer flask containing 25 ml. of 4 per cent. boric acid and titrated to the methylene blue-methyl red end point with 0.03 N HCl. This fraction is most accurately called hydrolyzable reduced nitrogen. Since no ammonium nitrogen was detected in the original water extract by the vacuum distillation method of PUCHER *et al.* (20), this fraction is probably



entirely amide nitrogen and will be so designated in this paper although specific amides were not isolated.

The difference between the water-soluble nitrogen and its amide-nitrogen fraction is, in the absence of both ammonium and nitrate ions, probably principally amino nitrogen and will be so designated in this paper although no specific determinations were made of this group. The amino-nitrogen linkage is not readily hydrolyzed by weak acid or alkaline solutions.

Protein nitrogen was calculated as the difference between total and water-soluble nitrogen since the usual water-soluble proteins are rendered insoluble in this procedure. Copper was determined by a colorimetric-carbamate method (10) after incinerating a 5-gm. sample in a platinum dish for 7 hours in a muffle oven at 450° C. and dissolving the ash in 6 N HCl.

Oil was determined in the flaked vacuum-dried samples of kernels by extraction with petroleum ether. Since it was impossible to flake the August samples thinly enough, the extracted meal was ground to 60 mesh and re-extracted, and the total oil was calculated after appropriate correction for loss of material in grinding.

## Results

### LEAVES

Since each determination represents the analysis from one composite sample per plot, the data could be subjected to statistical treatment by analysis of variance. The significance of the mean differences and seasonal interactions obtained were thus tested. Only the averages of the two determinations of each constituent per treatment are presented.

The data on the leaf composition are given in table I. Although the average milligrams of dry matter per leaf was higher for the deficient trees than for the normal ones, this difference is not significant in view of the high variability between readings. The deficient leaves, however, were lower than the normal ones in percentage of dry matter at all sampling periods. Leaves from deficient trees tended to have a lower percentage of reducing sugar than those from normal trees, a statistically significant but not outstanding difference. Non-reducing sugars remained rather constant in both types of leaves and on all three dates. There were highly significant differences, however, in the starch content of the leaves, particularly in the middle of August when formation of oil occurred in the fruit (21). At that time, the percentage of starch in the normal leaves was about 15 times that in the deficient ones. The acid-hydrolyzable polysaccharides, other than starch, tended to be somewhat higher in the deficient than in the normal leaves at the first and third sampling periods; but the difference lacks statistical significance, and the physiological significance of this conglomerate fraction is uncertain.

The outstanding difference in the nitrogen fractions was the large amount of protein nitrogen contained in the deficient leaves on both a percentage and a per-leaf basis. The differences in soluble nitrogen per leaf (sum of

TABLE I

COMPOSITION OF LEAVES FROM COPPER-DEFICIENT AND NORMAL TUNG TREES IN MG. PER LEAF AND PERCENTAGES

	JUNE 28				AUGUST 15				OCTOBER 23			
	DEFI- CIENT	NORMAL	DEFI- CIENT	%	NORMAL	DEFI- CIENT	%	NORMAL	DEFI- CIENT	%	NORMAL	DEFI- CIENT
Dry matter .....	mg.	mg.			mg.	mg.		mg.	mg.		mg.	mg.
Carbohydrates:	1125	887		37.5	43.8	1267	40.8	43.7	1477	38.7	1330	44.5
Reducing sugar .....	43.7	44.6		3.88	5.03	64.9	5.12	5.27	67.0	4.54	77.9	5.86
Non-reducing sugar .....	45.0	41.8		4.00	4.71	57.3	4.52	4.44	64.6	4.37	58.7	4.41
Starch .....	9.5	11.6		0.84	1.31	1.8	0.14	2.14	8.98	0.61	23.8	1.79
Polysaccharides* .....	66.2	43.8		5.88	4.93	57.5	4.54	4.56	83.1	5.63	71.5	5.38
Nitrogen fractions:												
Total .....	35.9	19.2		3.19	2.16	34.3	2.71	2.03	34.5	2.33	24.0	1.80
Protein .....	33.4	18.1		2.97	2.04	31.9	2.52	1.91	31.4	2.12	22.1	1.66
Amide .....	0.56	0.31		0.04	0.03	2.01	0.16	0.49	0.47	0.03	0.30	0.02
Amino .....	1.95	0.80		0.17	0.09	0.34	0.03	0.07	2.61	0.18	1.71	0.13
Copper† .....	3.20	5.45		2.84	6.14	4.84	3.8	6.9	9.63	6.52	16.13	12.13

\* Acid-hydrolyzable residue from starch determination.

† Weight per leaf expressed in gammas and concentration in p.p.m.

the amide and amino fractions) were of the same order. An increase from June to August in the amide form of nitrogen was found in both normal and deficient leaves but was especially marked in the deficient leaves, where there was a concomitant marked decrease in amino nitrogen.

The copper content was always lower in the deficient leaves but increased during the season in both normal and deficient leaves. This was in accord with field observation that the leaf-deficiency symptoms tend to disappear toward the end of the growing season.

#### FRUIT AND ITS COMPONENT PARTS

The data on the analysis of the kernels are presented in table II and on the hulls in table III. These data, together with the data on the analysis of the shells, were combined in table IV to give the composition of the fruit as a whole. The shells were included with the kernel fraction at the first sampling period because of the difficulty of mechanical separation at this stage. At the other periods, the shells were removed from the kernels and analyzed separately. Since the composition of the shells was found to be quite similar to the hulls, the data for this fraction are not presented here except as included in the calculation of the composition of the whole fruit.

#### KERNELS

In the June samples, which consisted of the whole tung nuts (kernels plus shells) and which were taken prior to the initiation of oil synthesis, there were no significant differences in dry weight or percentage of dry matter between the normal and deficient nuts (table II). In August the dry weight of the kernels of fruits from deficient trees was about 60 per cent. of what it was for normal trees. At maturity the dry weight of the kernels from deficient trees was 78 per cent. of that for the normal trees.

In June there was a significantly higher percentage content of sucrose and of starch but a lower amount of residual polysaccharides in the nuts from deficient trees than there was in those from normal trees. By August reducing sugar and starch had disappeared, and the differences in non-reducing sugar and polysaccharides are of doubtful significance. While starch continued to be absent from the kernels at maturity, reducing sugar accumulated again, particularly in the kernels from normal trees. Polysaccharides were likewise significantly lower, both on a percentage and a milligram basis, in the kernels from the deficient trees than they were in those from normal trees at the last date. In contrast to the leaf condition at the first sampling period, there were no significant differences in amount of either total or protein nitrogen between the two sets of nuts at the June sampling period. While the differences in the carbohydrate fractions in the nuts from deficient and normal trees were the opposite of those found in the leaves at this sampling period, the relationships of the amide and amino nitrogen fractions of the nuts were similar to those of the leaves. At the second date the nitrogen content in milligrams of the kernels from deficient

TABLE II  
COMPOSITION OF KERNELS FROM COPPER-DEFICIENT AND NORMAL TUNG TREES IN MG. PER FRUIT AND PERCENTAGES

	JUNE 28*				AUGUST 15				OCTOBER 23			
	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL
Dry matter .....	mg.†	3978	%	17.9	mg.†	1814	%	28.9	mg.†	8503	%	96.7
Carbohydrates:												
Reducing sugar .....	81.9	67.2	2.06	1.69	None	None	None	None	5.7	22.0	0.09	0.26
Non-reducing sugar .....	484.5	306.1	12.18	7.69	98.1	98.1	5.41	4.02	444.3	564.6	6.7	6.64
Starch .....	58.3	20.1	1.46	0.50	None	None	None	None	None	None	None	None
Polysaccharides†	320.1	428.5	8.04	10.77	68.9	68.9	3.80	3.85	392.2	692.4	5.91	8.14
Nitrogen fractions:												
Total .....	69.8	63.0	1.75	1.58	70.5	70.5	3.89	3.41	224.1	201.0	3.40	2.36
Protein .....	33.6	35.5	0.84	0.89	43.4	43.4	2.39	2.27	184.4	163.2	2.78	1.92
Amide .....	12.8	9.5	0.32	0.24	3.8	3.8	0.21	0.15	13.2	1.2	0.20	0.01
Amino .....	23.4	17.9	0.59	0.45	23.3	23.3	1.28	0.97	26.5	36.5	0.40	0.43
Oil .....	None	None	None	None	358.8	358.8	19.8	20.4	369.4	557.9	55.7	65.6
Copper‡	21.4	36.6	5.38	9.21	5.0	5.0	2.73	8.54	20.8	80.3	3.14	9.4

\* Including shells.

† Acid-hydrolyzable residue from starch determination.

‡ Four to five kernels per fruit.

§ Weight per fruit expressed in gammas and concentration in p.p.m.

trees was less than that of kernels from normal trees; there was a higher percentage of nitrogen, particularly soluble nitrogen, in the former. At maturity, however, although the percentage of total nitrogen was high, the milligram content per kernel was not significantly higher in the kernels from the deficient trees than in those from the normal trees because of their low dry weight.

No oil was found in June; but oil formation was well under way at the time of the August sampling, and about 10 per cent. of the final oil content on the per-fruit basis had already been formed. Since the percentage of oil was then about equal in kernels from normal and deficient trees, total oil per fruit was in about the same proportion as dry weight. The October samples of kernels from the deficient trees had a markedly lower percentage of oil than samples from normal trees, and the total amount of oil in the fruit from deficient trees was only about two-thirds that from the normal trees.

#### HULLS

The hulls (table III) of fruit from deficient trees tended to be lower in percentage and amount of dry matter than were the hulls from normal trees at all three sampling dates. The differences had statistical significance at approximately the 0.05 level on a weight-per-hull basis, and on a percentage basis they were significant for the first two sampling dates. At the first sampling date there were no significant differences in the sugar fractions in the hulls (table III), but the starch and other polysaccharides were lower on a percentage basis in the hulls from deficient trees than in those from normal trees. The sugar and starch content of the hulls in August was not affected significantly by copper deficiency. On a percentage basis, the mature hulls of fruits from deficient trees showed no differences in sugar and starch content from hulls of fruit from normal trees. The hulls from deficient trees were smaller than those of normal fruit and therefore had less of these carbohydrates on a milligram basis, but the differences were not significant. The residual polysaccharides, however, were significantly lower in the hulls from deficient trees than they were in the hulls from normal trees on both a percentage and a milligram basis at the three sampling dates.

In contrast to the carbohydrate data, all fractions of nitrogen were much higher in the hulls from the deficient trees than in those from normal trees. The differences were of very high order of significance on both percentage and per-hull bases. On the first two dates about 50 mg. of the total nitrogen per hull was in water-soluble compounds in the hulls of fruit from deficient trees in comparison with about 15 mg. of such nitrogen in the normal trees. The amounts on the last sampling date were 12.0 and 3.5 mg., respectively.

#### FRUIT

The trends in composition of the whole tung fruit generally follow the trends in the hulls. The differences in dry weight per fruit and in per-

TABLE III  
COMPOSITION OF HULLS FROM COPPER-DEFICIENT AND NORMAL TUNG TREES IN MG. PER HULL AND PERCENTAGES

	JUNE 28				AUGUST 15				OCTOBER 23			
	DEFI- CIENT	NORMAL	DEFI- CIENT	NORMAL	DEFI- CIENT	NORMAL	DEFI- CIENT	NORMAL	DEFI- CIENT	NORMAL	DEFI- CIENT	NORMAL
Dry matter .....	mg. 11844	mg. 12309	% 23.40	% 25.10	mg. 13338	mg. 15769	% 22.75	% 24.04	mg. 8489	mg. 11478	% 81.26	% 81.30
Carbohydrates:												
Reducing sugar .....	339.3	409.2	2.86	3.32	401.6	429.1	3.01	2.72	29.6	60.0	0.34	0.52
Non-reducing sugar .....	685.5	679.6	5.79	5.52	613.5	591.1	4.60	3.75	Trace	Trace	Trace	Trace
Starch .....	64.3	96.6	0.54	0.78	71.6	77.2	0.54	0.49	48.8	62.5	0.57	0.54
Polysaccharides* .....	904.7	1270.3	7.64	10.32	842.9	1066.2	6.32	6.76	724.8	967.3	8.54	8.43
Nitrogen fractions:												
Total .....	171.8	89.2	1.45	0.72	179.5	86.4	1.34	0.55	67.9	34.8	0.80	0.30
Protein .....	120.9	73.9	1.02	0.60	125.9	73.4	0.94	0.46	55.9	31.3	0.66	0.27
Amide .....	16.9	3.7	0.14	0.03	15.2	2.7	0.11	0.02	Trace	Trace	Trace	Trace
Amino .....	34.4	11.5	0.29	0.09	38.4	10.3	0.29	0.06	Trace	Trace	Trace	Trace
Copper† .....	25.6	64.9	2.2	5.3	31.8	45.0	2.4	2.8	18.0	42.3	2.1	3.7

\* Acid-hydrolyzable residue from starch determination.

† Weight per hull expressed in gammas and concentration in p.p.m.



centage of dry matter between fruit from normal and deficient trees were consistently significant throughout the season, being lower in the fruit from deficient trees.

The reducing sugar data showed no significant differences. Non-reducing sugar in the fruit from deficient trees was significantly higher than normal on a percentage basis, the difference becoming less as the season advanced. Differences in starch were not significant while the residual polysaccharides were consistently higher in the fruit from normal trees on a per-fruit basis and also on a percentage basis except for the August date.

As would be anticipated from the trends observed in the hulls and kernels, total nitrogen was higher on both weight-per-fruit and percentage bases in fruit from deficient trees than in fruit from normal trees.

Since the protein content of the fruit from deficient trees (table IV) was considerably greater than that from normal trees, and since proteins are not normally translocated in the elaborated form, it would seem that the process of translocation of soluble nitrogenous compounds to fruit had not been interfered with and that low copper content may have increased the synthesis of proteinaceous compounds within the fruit.

### Discussion

The most outstanding observation made in this work is that nitrogen metabolism was abnormal in the leaves and the fruit from copper-deficient tung trees. In this study a higher percentage of total nitrogen was always present in copper-deficient than in normal tissues at the same sampling period, which condition was associated with less starch in the leaves and less of other polysaccharides in the fruit parts. The synthesis of complex nitrogenous substances would account for the reduction in these carbohydrates, since such compounds are formed at the expense of part of the carbohydrate reserves. However, a study of the rate of apparent photosynthesis (18) in the copper-deficient tung leaves showed that it was only about one-fourth that of normal leaves. Thus the subnormal rate of apparent photosynthesis of deficient leaves, as well as the utilization of carbohydrates in protein synthesis, may account for the low carbohydrate reserves. Several factors could thus contribute to the final result, for a direct relation between synthesis of proteins and respiration has been observed (9). This positive relation would tend to increase the amount of carbon dioxide respired and thus to reduce the apparent rate of photosynthesis.

The existence of a relationship of nitrogen to copper deficiency has long been recognized in Florida citrus trees. FLOYD (12) refers to the relationship between heavy applications of nitrogenous fertilizers and the development of the symptoms of a disease known as "ammoniation" or exanthema. More recently, CAMP and FUDGE (8) have suggested that this disorder may be caused by an improper copper-nitrogen balance. The data referred to (13) are limited, but the suggestion is in agreement with the data on tung reported here.

TABLE IV

COMPOSITION OF WHOLE FRUIT FROM COPPER-DEFICIENT AND NORMAL TUNG TREES IN MG. PER FRUIT AND PERCENTAGES

	JUNE 28				AUGUST 15				OCTOBER 23			
	DEPT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL	DEFT- CIENT	NORMAL
Dry matter .....	mg.	16287	%	22.9	mg.	23055	%	28.06	mg.	25566	%	87.60
Carbohydrates:	15823		21.0		18447		25.16		20104		86.96	
Reducing sugar .....	421.1	480.8	2.66	2.95	419.7	446.3	2.27	1.33	44.9	87.7	0.22	0.34
Non-reducing sugar .....	1170.0	991.2	7.39	6.08	739.9	736.2	4.01	3.19	460.4	580.6	2.29	2.27
Starch .....	122.6	116.8	0.77	0.72	71.6	77.2	0.39	0.33	61.3	78.2	0.30	0.30
Polysaccharides* .....	1225.0	1699.0	7.74	10.43	1333.0	1634.0	7.23	7.09	1575	2119.0	7.83	8.29
Nitrogen fractions:												
Total .....	241.6	152.2	1.53	0.93	270.7	204.1	1.47	0.88	330.0	256.0	1.64	1.00
Protein .....	154.1	109.4	0.97	0.67	185.6	155.1	1.01	0.67	272.4	213.3	1.35	0.83
Amide .....	29.7	13.3	0.19	0.08	20.2	8.0	0.11	0.03	14.9	2.0	0.07	0.08
Amino .....	57.8	29.5	0.36	0.18	64.8	41.2	0.35	0.18	42.7	40.7	0.21	0.16
Copper† .....	49.8	101.6	3.15	6.2	45.9	91.1	2.5	3.9	53.8	150.5	2.7	5.9
Oil .....	None	None	None	None	358.8	610.2	1.95	2.65	3694.0	5579.0	18.37	21.82

\* Acid-hydrolyzable residue from starch determination.

† Weight per fruit expressed in gammas and concentration in p.p.m.

One possible explanation that may be advanced for this relationship is that the excessive growth induced by heavy nitrogen applications results in a dilution of the available copper in the plant. This hypothesis, however, is not in accord with the fact that, as is evident from the description of this disorder in a number of plants (3), an important and characteristic symptom of the disease is cessation of terminal growth and a pronounced "dieback." An extensive examination of this feature was made on young tung trees (14) and the additional unpublished data show conclusively that even in the early stages of a copper deficiency induced by heavy nitrogen applications there is no correlation of linear growth to severity of the disease. Under these conditions where copper was a limiting factor, the heavier nitrogen applications failed to increase linear growth, yet they greatly increased incidence of copper-deficiency symptoms.

A second possible hypothesis, based on the fact that this dieback is such a prominent symptom of this deficiency, would attribute the accumulation of nitrogen in the deficient leaf to proteolysis in the terminal leaves and bud, followed by translocation of the nitrogen compounds to the midshoot leaves. The cessation of terminal growth, resulting from a deficiency of copper for meristematic growth, would, moreover, lead to an accumulation in these midshoot leaves of the nitrogen absorbed from the soil. An analogous mineral deficiency primarily affecting meristematic growth is that of boron. When the supply of boron is removed from the plant, an accumulation of carbohydrates follows (16) and probably a pronounced proteolysis, since there is a decrease in protein and an increase in the soluble nitrogen fractions (7). Such results would be expected where the ability of the cells to divide and synthesize new protoplasm is affected. In copper deficiency, on the other hand, no such accumulation of carbohydrates takes place in the midshoot leaves, and there is, moreover, an increased synthesis of complex nitrogen compounds. It is evident, therefore, that no simple explanation based on growth alone will be satisfactory in interpreting the data pertaining to copper deficiency. A satisfactory hypothesis must account for the increased synthesis of complex nitrogenous compounds while the normal growth of the plant is disrupted.

In view of the fact that copper is required by the tung plant in such small amounts, this element most probably functions through the enzyme system of the plant. The existence of enzymes containing copper as a prosthetic element has been demonstrated for the ascorbic acid oxidase and the mono- and polyphenol oxidases (17).

The phenomenon of the inactivation of copper fungicides by proteinaceous substances (15) may be analogous to the effect of nitrogen in increasing the severity of the symptoms of copper deficiency of tung (14). A high protein content within the plant may inactivate the absorbed copper. The data obtained in this study show that an abnormally high protein content exists in copper-deficient tung leaves. Further work is in progress on the nature of the copper/protein relationship in tung.

The data on leaf composition could be taken to indicate an active synthesis of complex compounds of nitrogen, for example protein, in the deficient leaf with an accumulation of the intermediate forms such as amino acids, polypeptides, and amides. The data indicate that a highly abnormal amide-nitrogen condition existed in the copper-deficient leaves in August. The amide nitrogen is regarded by some workers (22) as acting as an intermediary in protein synthesis and by others (19) as a manifestation of abnormal nitrogen metabolism such as is found in ammonium detoxification.

The principal effect in the tung kernels associated with copper deficiency was a decrease in the formation of oil. The yield of oil from copper-deficient trees was only two-thirds of the yield from normal trees because of the smaller size of the fruits and the lower percentage of oil in them. Oil synthesis, which takes place during the middle and latter part of the growing season, is essentially a reduction of carbohydrate (21). A reduction in copper supply may play a relatively direct rôle by lessening enzymatic action in the formation of oil or an indirect rôle by preventing an accumulation of sufficient carbohydrate reserves for maximum oil formation in copper-deficient trees. This decrease in oil is of great economic significance in tung culture and it emphasizes the importance of supplying sufficient copper to trees growing on soils in which this element may be deficient.

### Summary

The composition of the leaves and fruit of copper-deficient tung trees was compared with that of normal trees in the same orchard at different times during the growing season, in order to determine how the metabolic processes were affected.

There were no significant differences between leaves from normal and copper-deficient trees in the percentage of non-reducing sugar; but the percentage of reducing sugar in deficient leaves was low and that of starch was outstandingly low, a condition indicating either a decrease in formation or an increase in utilization of reserve carbohydrates in such tissues, or both.

The total nitrogen of deficient leaves was always higher on both milligram and percentage bases than that of normal leaves at the same sampling date. This difference was due in large measure to the abnormally high amount of water-insoluble nitrogen found in deficient leaves. The accumulation of this elaborated nitrogen fraction was a characteristic symptom of copper deficiency and indicated the importance of copper in the nitrogen metabolism of the normal plant. It appears likely that in the absence of sufficient copper the plant forms abnormal amounts of complex nitrogen compounds at the expense of the carbohydrate reserves.

The greatest effect of the deficiency on the fruit was the failure of the kernel to attain normal size and to synthesize a normal amount of oil. This decrease in oil production is of considerable economic importance on soils low in copper.

The composition of the hulls of the tung fruit of normal and deficient trees was in general analogous to that of the leaves.

The physiology of the copper/nitrogen interrelationship was examined. Growth alone cannot account for the effects observed, and, therefore, the exact rôle of copper in nitrogen metabolism must be given further study.

The authors are indebted to MR. GUILFORD T. SIMS for the copper analyses reported herein.

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## EFFECT OF OXYGEN AND SODIUM THIOGLYCOLLATE ON GROWTH OF RICE

CH'WAN-KWANG LIN

(WITH ONE FIGURE)

Rice is the plant upon which a great part of the world's population depends for its principal food supply (42). In most subtropical regions, it outyields any other crop per unit area of land. However, there are some obvious disadvantages of rice-farming which arise from its cultivation under flooded conditions. Irrigation is expensive and in many places impossible. In rice countries, a short period of drought may result in a disastrous famine. Furthermore, the paddy rice field is the chief breeding ground for mosquitoes which spread malaria.

For thousands of years rice has been known to be well adapted to a semi-aquatic habitat. It is true that there is "upland rice," but according to JONES (23), in the orient and elsewhere, the best upland varieties give lower yields when grown on upland than when irrigated. VLAMIS and DAVIS (41) showed that the growth of rice was consistently better in submerged soil than in drained soil, even though the latter is watered every day. JONES' experiments (22) indicate that the average yield of rice over an eight-year period increases with the increase of depth of submergence to six inches.

On the other hand, there is no evidence that throughout its growing period the rice plant has a water requirement especially higher than that of dry land plants. BRIGGS and SHANTZ (3) give 710 as the average value for the water requirement of rice which is not much higher than that of rye and considerably lower than those of many legumes and grasses. LEATHER'S (27) value for rice is 811, which is about the same as those for flax and chickpea. The available data on water requirements of rice are not entirely adequate, and the values given are probably too high, as they have been obtained under conditions which are unfavorable for the production of dry matter of this plant. It is evident that the rice plant would hardly require more water for direct consumption than could be adequately supplied by a well-drained soil. The effect of the superfluous water on rice growth, then, must be indirect.

Considerable is known as to why a semi-aquatic plant like rice can flourish in submerged soil (6, 32, 38), but few attempts have been made to find out why it cannot flourish in drained soil. The main emphasis of the present investigation is placed on the latter phase of this problem.

This paper reveals that excessive oxygen is not injurious to the rice plant, and a reducing organic substance, sodium thioglycollate, is favorable for its growth. The physiological action of sodium thioglycollate is apparently related to the iron and nitrogen nutrition.

## Experimentation

### MATERIALS

The rice seeds used in all the experiments reported in this paper are of 1944 California grown early variety, Colusa, obtained through the courtesy of Mr. J. W. JONES of the United States Department of Agriculture. The wheat seeds used in one of the experiments are of 1943 New York grown Yorkwin variety.

High grade reagent chemicals were employed. Nitrogen was from a commercial tank which contained about 0.2 per cent. oxygen. While all the glassware was thoroughly cleaned before use, no special attention was given to the quality of glass. Ordinary distilled water was used in all the experiments.

### OBJECT, PROCEDURES AND RESULTS

CLOSED CHAMBER WATER CULTURE OF SPROUTS ATTACHED TO THE SEEDS.—SHIVE (35) has recently demonstrated for solution culture that, while the growth of tomato and barley is promoted by high concentrations of oxygen, soybean is injured by a concentration of oxygen higher than that of the atmosphere. In the light of this work, it was thought that rice might be even less tolerant than soybean, thus favoring its growth in water-logged soil. Although TAYLOR (38) and VLAMIS and DAVIS (40) have already carried out experiments to study the effect of various oxygen concentrations on the growth and respiration of young rice seedlings, their experiments have been carried out in darkness, and the highest oxygen concentration employed was only 20.8 per cent. The possibility remains that when rapid photosynthesis is taking place, the internal concentration of oxygen may be raised. Experiments were therefore designed to determine the response of rice to a wider range of oxygen concentrations under illumination.

Uniform seeds were selected and disinfected with a saturated solution of calcium hypochlorite for 20 minutes. They were allowed to germinate on moistened filter paper in Petri dishes in an incubator at 28° C. for rice and 21° C. for wheat.

For studying the effect of constant oxygen concentrations in closed chambers fruit jars of half-liter capacity were used. The jars were filled with glass beads and distilled water to a depth of about 1.5 inches. A hole was drilled through the cover of each jar to accommodate a rubber stopper through which was passed a gas-inlet capillary tube which extended nearly to the bottom of the jar and a gas-outlet, inverted U-shaped glass tube connected outside to a small vial to provide a water seal. The inlet tube was bent away from the outlet tube to assure a thorough circulation of the gas in the jar.

In each jar, 25 germinated seeds were supported at the surface of the water by glass beads. At the start of the experiment, about 3 liters of gas mixture were flushed through each chamber within 30 minutes, and subsequently the rate of the gas flow was adjusted at about 5 ml. per minute

throughout the experimental period. Gas mixtures were prepared by displacing definite proportions of tap water from a 20- or 40-liter bottle with commercial oxygen where the natural air was employed. No effort was made to add CO<sub>2</sub> to the artificial gas mixtures nor to remove CO<sub>2</sub> from the air.

Illumination was provided by two fluorescent lamps placed two feet above the chambers. For darkness, the fruit jars were completely wrapped with black paper. The temperature at different times during the experimental period varied from 23° to 27° C.

The time allowed for the germination of the seeds in the incubators before the start of the gas treatment was 74 hours in Experiment I, 56 hours in Experiment II, and 128 hours in Experiment III.

It was noticed that after the sprouts were illuminated, the shoots turned green rapidly under an oxygen concentration of 5.2 per cent. or above.

TABLE I\*

EFFECT OF VARIOUS OXYGEN CONCENTRATIONS ON THE GROWTH OF RICE SEEDLINGS UNDER CONTINUOUS ILLUMINATION FOR 100 HOURS

PERCENTAGE OXYGEN	DRY WEIGHTS OF 25 SEEDLINGS			SHOOT/ROOT RATIO
	SHOOT	ROOT	TOTAL	
%	mg.	mg.	mg.	
0.2	35.3	11.3	46.6	3.13
1.2	37.5	15.4	52.9	2.44
5.2	47.7	32.4	80.1	1.47
10.2	58.8	40.3	99.1	1.46
20.8	58.6	43.0	101.6	1.36
50.1	71.1	47.5	118.6	1.50

\* Experiment I.

There was still no perceptible greening of the shoots subjected to 0.2 per cent. oxygen as long as 24 hours after illumination. Only slight greening occurred in the shoots in 1.2 per cent. oxygen chambers during the same period. By the end of the experimental periods, however, all the shoots were green although of lighter shades at lower oxygen concentrations. Rice and wheat were similar in their responses. These results are in agreement with the findings of others that chlorophyll formation is retarded under conditions of oxygen deficiency.

At the end of the experimental period, all the jars were disconnected from the gas system at the same time and placed in an ice chest to stop the growth. The seed was then removed from each young seedling. The seedlings were transferred to a Petri dish containing distilled water, and every bit of detachable tissue was removed from the basal portion of the shoots. The roots and the shoots were carefully separated and transferred to separate crucibles. They were dried at 105° C. for 24 hours and their dry weights finally determined.

TABLE II\*

EFFECT OF VARIOUS OXYGEN CONCENTRATIONS ON THE GROWTH OF SEEDLINGS OF RICE AND WHEAT UNDER CONTINUOUS ILLUMINATION; DURATION: 60 HOURS FOR WHEAT AND 100 HOURS FOR RICE

PERCENTAGE OXYGEN	TOTAL DRY WEIGHT OF 25 SEEDLINGS		SHOOT/ROOT RATIO	
	WHEAT	RICE	WHEAT	RICE
%	mg.	mg.		
0.2	88.1	34.4	2.91	5.61
1.2	125.0	47.2	3.51	3.97
5.2	159.1	59.3	3.07	3.42
10.2	198.6	81.5	3.70	1.73
20.8	210.0	83.7	3.06	1.51
50.1	226.3	100.9	3.12	1.43

\* Experiment II.

Data on the amounts of growth and shoot/root ratios on the basis of dry weight are shown in tables I, II, and III, respectively, for the aforementioned three experiments.

It is seen that the growth of both shoots and roots of rice seedlings, as well as those of wheat, in general increases with an increase of oxygen concentration up to 50.1 per cent. oxygen, the highest employed in the experiment. Particularly remarkable is the fact that the increase of oxygen concentration results in a higher increase of root growth of illuminated rice seedlings than that of either unilluminated rice seedlings or illuminated wheat seedlings, as shown by the difference in the shoot/root ratios.

The results pertaining to the growth of the rice seedlings in relation to the oxygen concentrations in darkness generally agree with those of TAYLOR (38). The suppressive effect of oxygen on the growth of rice shoots such as shown in one of the experiments of VLAMIS and DAVIS (40) is not here observed. The latter investigators, however, based their observations on measurements of the fresh weight. Possibly there is a difference in the

TABLE III\*

EFFECT OF VARIOUS OXYGEN CONCENTRATIONS ON THE GROWTH OF RICE SEEDLING UNDER CONTINUOUS ILLUMINATION AND DARKNESS, DURATION 108 HOURS

PERCENTAGE OXYGEN	TOTAL DRY WEIGHTS OF 25 SEEDLINGS		SHOOT/ROOT RATIO	
	LIGHT	DARKNESS	LIGHT	DARKNESS
%	mg.	mg.		
0.2	67.3	52.2	2.89	2.95
1.2	62.3	91.7	2.80	2.73
5.2	85.9	113.9	2.33	2.06
10.2	112.1	100.7	1.65	2.23
20.8	122.2	127.6	1.80	2.02
50.1	142.6	135.0	1.91	2.30

\* Experiment III.

percentages of dry matter of the rice seedlings grown under different oxygen tensions.

**SOLUTION CULTURE OF SEEDLINGS DETACHED FROM THE SEEDS.**—As will be discussed later, numerous investigators have observed peculiarities of the rice plant in its response to plant nutrients. Fertilization has always been one of the most difficult problems in rice culture. However, it has long been the experience of farmers and investigators that heavy application of organic matter is generally desirable (36). Ammonium sulphate is the only commercial fertilizer regularly used in some regions. Chlorosis due to iron deficiency commonly occurs on rice plants grown on dry land. It is highly suggestive that these facts might be related to the adaptation of the rice plant to a semi-aquatic habitat.

An attempt was made, by means of solution culture method, to find out if there were any inter-relationships among aeration, nitrogen nutrition,

TABLE IV

COMPOSITION OF THE SOLUTIONS USED IN THE SOLUTION CULTURE EXPERIMENT

INGREDIENTS	UNIT OF CONCN.	CONCENTRATION IN SOLUTION*							
		A	B	C	D	E	F	G	H
Ca(NO <sub>3</sub> ) <sub>2</sub>	m.mol	7.5	7.5	.....	.....	7.5	7.5	.....	.....
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	"	.....	.....	7.5	7.5	.....	.....	7.5	7.5
FeSO <sub>4</sub> · 7H <sub>2</sub> O	p.p.m.	5.0	.....	5.0	.....	5.0	.....	5.0	.....
Fe <sub>2</sub> (C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> ) <sub>3</sub>	"	.....	5.0	.....	5.0	.....	5.0	.....	5.0
CaSO <sub>4</sub>	m.mol	.....	.....	1.0	1.0	.....	.....	1.0	1.0
KH <sub>2</sub> PO <sub>4</sub>	"	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
MgSO <sub>4</sub>	"	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HSCH <sub>2</sub> COONa	p.p.m.	.....	.....	.....	.....	50.0	50.0	50.0	50.0

\* Micro-elements added to all the solutions as HOAGLAND'S supplementary A-Z solution.

iron nutrition, and the presence of a reducing substance, as measured by the growth response of the rice plant.

The rice seeds were sown on April 17, 1945, on quartz sand in a tumbler with the water level kept just at the surface. The tumbler was placed in an incubator for three days at 28° C. and then moved to a greenhouse. On May 4, the aerial part of the seedlings had attained a height of 10–12 cm. They were detached from the seed, rinsed with distilled water, and transferred to the previously prepared culture vessels.

Brown glass chemical bottles of 500-ml. capacity were used for culture vessels. The method of maintaining aeration and continuous flow of culture solutions was essentially that of GILBERT and SHIVE (12). Five-holed cork stoppers infiltrated with paraffin were used instead of rubber stoppers. The plant was inserted through a glass tube which passed through the central hole to the solution to produce a water seal. Two of the side holes were used for the inlet and the outlet of the solution and the other two for passage of gas. When the continuous flow of the solution was omitted, the

TABLE V  
FACTORS INCLUDED IN THE SOLUTION CULTURE EXPERIMENT

CULTURE SERIES NUMBER	SOLN. NUM- BER	SOURCE OF NITROGEN NUTRIENT	SOURCE OF IRON NUTRIENT	SUPPLE- MENT OF SODIUM THIOGLY- COLLATE	CONDITION OF NUTRIENT SOLUTION SUPPLY	CONDITION OF AERATION
1	A	$\text{Ca}(\text{NO}_3)_2$	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	Stagnant	Without
2	B	"	$\text{FeSO}_4$	-	"	bubbling
3	A	"	$\text{FeSO}_4$	-	"	Without
4	B	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	"	bubbling
5	A	"	$\text{FeSO}_4$	-	"	Air
6	A	"	"	-	"	bubbling
7	B	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	Continuous	Nitrogen
8	A	"	$\text{FeSO}_4$	-	flow	bubbling
9	B	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	Continuous	Without
10	A	"	$\text{FeSO}_4$	-	flow	bubbling
11	E	"	"	+	Continuous	Air
12	F	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	+	flow	bubbling
13	E	"	$\text{FeSO}_4$	+	Continuous	Nitrogen
14	F	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	+	flow	bubbling
15	C	$(\text{NH}_4)_2\text{SO}_4$	$\text{FeSO}_4$	-	Stagnant	Without
16	D	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	"	bubbling
17	C	"	$\text{FeSO}_4$	-	"	Without
18	D	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	"	bubbling
19	C	"	$\text{FeSO}_4$	-	"	Air
20	C	"	"	-	"	bubbling
21	D	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	Continuous	Nitrogen
22	C	"	$\text{FeSO}_4$	-	flow	bubbling
23	D	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	-	Continuous	Without
24	C	"	$\text{FeSO}_4$	-	flow	bubbling
25	G	"	"	+	Continuous	Air
26	H	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	+	flow	bubbling
27	G	"	$\text{FeSO}_4$	+	Stagnant	Nitrogen
28	H	"	$\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$	+	"	bubbling



two empty holes were sealed with paraffin. When the bubbling of gas was omitted, the two empty holes were plugged with a loose wett of cotton. When both the continuous flow of the solution and bubbling of the gas were omitted, all the four empty holes were plugged with cotton.

The rate of the bubbling of air or nitrogen was adjusted to about 10 ml. per minute; the air was from a compressed air line, and the nitrogen from

TABLE VI  
SUMMARY OF RESULTS OF SOLUTION CULTURE EXPERIMENT

CULTURE SERIES NUMBER	TOTAL GREEN WEIGHT	TOP/ROOT RATIO ON GREEN BASIS	TOTAL DRY WEIGHT	TOP/ROOT RATIO ON DRY BASIS	SYMPTOMS	FINAL PH
	<i>mg.</i>		<i>mg.</i>			
1	780	2.2	170	3.8	Chlorosis	5.7
2	584	2.0	117	3.6	"	5.7
3	901	1.8	157	3.5	"	5.8
4	871	1.8	149	3.2	"	5.8
5	977	2.1	184	3.6	"	5.7
6	978	1.6	156	3.0	None	5.1
7	842	1.6	134	3.1	"	5.5
8	1207	1.5	194	2.9	"	4.8
9	1156	1.5	187	2.9	"	5.3
10	1622	1.6	256	2.9	"	5.0
11	1577	1.2	277	2.3	"	6.2
12	1695	1.2	301	2.0	"	6.1
13	1436	1.3	237	2.3	"	6.2
14	1864	1.2	324	2.0	"	6.1
15	717	2.8	156	6.6	Tip-burn	3.7
16	1093	2.6	220	5.3	"	3.4
17	1189	2.8	232	6.0	"	3.3
18	1228	2.1	238	6.7	"	3.3
19	1330	2.9	236	5.8	"	3.3
20	859	3.7	154	4.8	Slight tip-burn	4.1
21	1230	2.0	201	3.8	" "	4.3
22	1332	2.6	237	5.0	" "	3.9
23	1022	4.4	207	7.6	" "	3.8
24	581	2.8	109	4.7	" "	4.1
25	1644	2.4	315	4.3	None	3.8
26	1282	1.8	223	3.9	"	4.2
27	1496	3.7	260	7.1	Very slight tip-burn	3.8
28	1309	2.9	258	5.1	None	4.0

a commercial tank. The flow of solution was adjusted to a rate of about one liter per day from a reservoir of 6-liter capacity under the pressure of nitrogen.

The eight nutrient solutions employed, differing in the sources of nitrogen and iron and presence or absence of sodium thioglycollate, had the compositions shown in table IV.

Twenty-eight sets of duplicate cultures involving five varying factors as shown in table V were carried out. The greenhouse temperature ranged from 25° to 32° C. during the experimental period.

Observations of the appearance of the plants were made from time to

time. On May 25, the end of a three-week experimental period, both fresh and dry weight of the root and top of each plant were determined separately. The determinations of the fresh weights of the roots were made immediately after blotting off the surface water with a piece of filter paper. Here the experimental error is inevitably large. A summary of the results is shown in table VI.

The outstanding results were as follows:

1. Plants supplied with nitrate nitrogen in stagnant solution developed severe chlorosis. This chlorosis was discernible as early as one week after the plants were placed in the solution. It always occurred on the newly formed upper leaves. The lower leaves remained green throughout the experimental period. The development of brown necrotic streaks which usually appeared near the margin of the chlorotic leaves was also noticed.

2. Plants supplied with ammonium nitrogen in stagnant solution developed severe tip-burn of the lower leaves. This symptom occurred rather suddenly in the third week of the experiment. The upper third to half of the lower leaves rolled up under high noon temperatures and dried rapidly. About two days later, this dried part turned straw-colored.

3. Chlorosis does not develop if the plants are continually supplied with a fresh nitrate-containing solution. Continuous flow of solution, however, does not completely remedy the tip-burn symptom of the ammonium-supplied plants.

4. When sodium thioglycollate is present in either the nitrate-containing or the ammonium-containing stagnant solution, the plants develop neither chlorosis nor tip-burn.

5. In the absence of sodium thioglycollate, the total amount of growth was in general higher with the ammonium nitrogen than with nitrate nitrogen. In the presence of sodium thioglycollate, the total amount of growth was about the same with either source of nitrogen. The increase of growth due to sodium thioglycollate was about 20 per cent. in the ammonium series while it was almost 100 per cent. in the nitrate series.

6. Nitrate nitrogen was more favorable for root development than ammonium nitrogen, inasmuch as the top-root ratio in the ammonium series was generally about twice as high as in the nitrate series.

7. No marked differences in growth were found between ferrous sulphate and ferric tartrate as the source of iron, nor between various amounts of oxygen supply.

The above generalizations are made on the assumption that the difference in the total osmotic concentration, calcium concentration, and sulphate concentration under the experimental conditions did not affect the results. Reservations should be made, however, that such an assumption might not be totally correct.

### Discussion

#### OXYGEN RELATIONS

It has long been known that rice seeds can germinate in water containing

very little or no oxygen (29, 37, 44). The possibility of the utilization of fermentation energy by the germinating rice embryo was intimated by TAKAHASHI (37) as early as 1905. Recently, TAYLOR (36) measured  $O_2$  intake and  $CO_2$  output of the germinating embryo of rice and wheat under various oxygen concentrations using a BARCROFT-WARBURG manometer. He demonstrated that with 20.8 per cent. oxygen the  $CO_2/O_2$  ratio was entirely characteristic of respiration both in rice and in wheat, but as the oxygen concentration dropped below 5 per cent., the fermentation activity in rice was 2 to 7 times as great as in wheat. Growth of the rice embryo under low oxygen concentration was thus correlated with the liberation of fermentation energy.

In closed chamber experiments with germinating seeds, all the previous investigations (29, 38, 40, 44) have observed that at least the root growth increases very consistently with an increase of the oxygen concentration up to 20.8 per cent. oxygen. It is here further shown that this is true up to 50.1 per cent. oxygen, even under continuous illumination. However, aeration does not seem to affect the increase of weight of the rice plant grown in a variety of nutrient solutions with the top of the plant exposed to the open air. On this point, the data of VLAMIS and DAVIS (41) and those of the writer agree fairly well. Inasmuch as neither bubbling air nor nitrogen through the solution gives a measurable difference of growth, it can be inferred that the rice plant is indifferent to the concentration of oxygen in the environment of the roots.

The rice plant appears, however, to be as susceptible to  $CO_2$  as any other plant. This was first discovered by VLAMIS and DAVIS (41) and promptly confirmed by CHANG and LOOMIS (5). A root rot disease of rice, called "mentek" in Java and "brusone" in Italy, has hitherto been ascribed to oxygen deficiency. COPELAND (7), after citing VAN DER ELST's experiment (9), stated: "The demonstration that want of oxygen can cause root rot is conclusive." The original experiments of VAN DER ELST, however, as well as that of BRIZI (4) were carried out under conditions which prevented the entry of oxygen and at the same time favored the accumulation of  $CO_2$ . In the light of the work of VLAMIS and DAVIS and that of CHANG and LOOMIS, it is more likely that the rice plant suffers from too much  $CO_2$  than from too little oxygen.

In 1940, RAALTE (32), by direct analysis of the gas obtained from the air spaces in the rice roots, has demonstrated very convincingly that the root depends entirely on the top for its oxygen supply. He found that there is a gradient of oxygen concentration in the root from 9-14 per cent. in the basal portion to 2.1-8.1 per cent. in the tip portion. Furthermore, he showed that the oxygen concentration in the roots is not lowered by growing the plant in OMELIANSKY's cellulose fermentation medium nor by bubbling commercial nitrogen through a nutrient solution. Strangely enough, according to RAALTE's results, photosynthesis does not cause an increase of the oxygen concentration in the roots. The writer's data also

indicate that illumination does not materially increase the amount of growth nor significantly alter the shoot/root ratio of the rice seedlings under low oxygen concentration.

#### MINERAL NUTRITION

GERICKE (11) in 1930 was able to grow eight-week-old, complete-nutrient-solution-grown rice seedlings to maturity without any further supplement of magnesium, sulphur, phosphorus, and calcium. Of the remaining three macro-elements, the requirement of nitrogen and iron seemed most striking. GERICKE used nitrate as the source of nitrogen, and he found it necessary to add iron as tartrate every three days in order to maintain normal growth. Incidentally, the literature on the mineral nutrition of rice is restricted largely to iron and nitrogen nutrition.

Comparative studies of nitrate and ammonium as the source of nitrogen for the rice plant began with the work of KELLNER in 1884 (26). KELLNER observed in his solution cultures that during the first three weeks the nitrate-supplied plants appeared sickly and the ammonium-supplied plants made almost twice as much growth as the nitrate-supplied plants on the basis of the height of the plants. Following this period, he found that the situation was reversed so that by the end of the 138-day experimental period the nitrate plants yielded more than the ammonium-supplied plants. His results also showed that the best growth throughout the experimental period was obtained by supplying both nitrate and ammonium nitrogen.

In soil cultures, application of nitrate usually fails to bring about a significant increase of yield (8, 22, 25, 30, 39, 43) and is likely to induce chlorosis (8, 25, 28, 43). Application of ammonium nitrogen, however, almost always results in good growth. The notion has become widespread that ammonium nitrogen is indispensable for the growth of rice.

Explanations of the undesirable effect of nitrate nitrogen have been attempted by several investigators; NAGAOKA (30) suggested the possibility of transformation of nitrates into asparagin in the nitrate-supplied plants. Analyses carried out by DAIKUHARA and IMASEKI (8), however, showed that there was no significant difference in the sugar content between the plants of both upland and lowland varieties fertilized with ammonium sulphate and those fertilized with sodium nitrate. The latter investigators attributed the ineffectiveness of nitrate as a fertilizer to the loss of nitrate nitrogen in paddy soil by leaching, denitrification, and the toxic effect of nitrite resulting from denitrification. Although DAIKUHARA and IMASEKI found that the water-logged soil in their laboratory flask test contained 33-70 p.p.m. nitrite at its peak, KELLY (25) showed that in no instance did nitrite accumulate to an extent greater than 2 p.p.m. of the irrigating water. BARTHOLOMEW (1), from the results showing very spasmodic production of nitrite due to denitrification in his experiments, denied the contention that the production of nitrites from nitrates was the cause for the failure of nitrates to produce good yields of rice. KELLY also indicated that the yields of rice from the single application of nitrate were greater two times out of three than the

yields from the repeated applications, an observation which ruled out in large part the operation of leaching as a factor in nitrate inefficiency. KELLY finally thought it possible that rice, which has been grown for centuries under conditions that largely exclude the formation of nitrates, had in a large measure lost the nitrate-reducing enzymes which were necessary in the process of nitrogen metabolism with nitrate as the raw material. That the rice plant can grow to maturity in solution culture with nitrate nitrogen under the conditions of such experiments as carried out by GERICKE (11) and KAPP (24) again rendered KELLY's postulate untenable.

The first attempt to relate the iron-deficiency symptom, chlorosis, to nitrate fertilizer and soil alkalinity was made by WILLIS and CARRERO (43). In the soils they used, the application of ammonium sulphate or calcium nitrate failed to bring about any marked change in the soil pH after the plant growth. Yet the plants developed chlorosis where nitrate was applied. They were inclined to the view that possibly the production of the basic residues, other than that affecting pH or the absorption of nitrates, produced in the soil a condition that presented the absorption of iron, or in the plant a condition which prevented the utilization of iron for the development of chlorophyll.

Chlorosis of upland rice in calcareous soil was studied by GILE and his co-workers in a series of experiments (13, 18). They have produced a body of evidence indicating iron deficiency as the cause. The fact that spraying with soluble iron compounds only results in localized greening on the leaves and that the lower leaves usually contain a higher amount of iron than the upper leaves led them to the belief that the iron is immobilized in the plant.

On the other hand, emphasis has been placed on the apparent iron injury by STURGIS (36) who attributed root discoloration to the incrustation of iron. A significant observation made by STURGIS was that the application of organic matter which induced a very reducing condition in the soil prevented root discoloration.

Judging from the symptoms, there is little doubt that the chlorosis of the plant growing in stagnant nitrate-containing solution is due to iron deficiency. Just how the nitrate interferes with the absorption or assimilation of iron is not definitely known at present. It is interesting to point out that the reducing substance, sodium thioglycollate, prevents chlorosis despite the fact that it raises the pH to a higher level.

The tip-burn symptom developed on the plants supplied with ammonium sulphate as the sole source of nitrogen has also been previously observed by KELLNER (26) and fully described by ESPINO (10). This could be due to the high top-root ratio resulting from the inhibition of root growth by low pH. On this basis, the prevention of tip-burn by sodium thioglycollate and the disappearance of the symptom under field conditions could be easily explained. However, the writer's results do not indicate a perfect correlation between tip-burn, high top/root ratio, and low pH, particularly in case of continuous flow of the nutrient solution. Furthermore, he is not at



all sure that the high top/root ratio observed is the direct cause of tip-burn, as SETHI's extensive measurements (34) have shown that a top/root ratio as high as 10 on a dry-weight basis is not uncommon among vigorous field-grown plants.

#### POSSIBLE RÔLES OF SODIUM THIOLYCOLLATE

Sodium thioglycollate, or mercaptoacetate, possesses several properties of biological interest. It forms a soluble red-purple product with iron in a very alkaline medium, a reaction which has been utilized in the colorimetric determination of traces of iron (33). As a rather stable effective reducing agent, sodium thioglycollate is widely used to maintain a low oxidation-reduction potential in a medium for the cultivation of anaerobic bacteria (2). Its sulphydral component has been shown by HAMMETT (19) to be an active "proliferation stimulus" (20) which promotes cell division in root tips. Sodium thioglycollate is also known to be an effective antidote for mercurial disinfectants (21, 31). Whether any of these known properties is involved in the prevention of chlorosis of the rice plant in a stagnant nitrate-containing solution and of tip-burn in an ammonium-containing solution is not immediately clear.

#### POSSIBILITY OF UPLAND CULTIVATION OF PADDY RICE

It is premature to predict the applicability of the findings reported in this paper to the dry land cultivation of paddy rice. It is shown, however, that a high oxygen concentration is beneficial to the germinating embryo and good aeration is not harmful to the growing plant; chlorosis induced by the application of nitrates can be remedied; and nitrate, itself, is a suitable source of nitrogen for the rice plant. Thus there is a good possibility that through the application of a substance playing the same rôle as does sodium thioglycollate, rice may give as high yields on dry as on submerged land, provided enough water is available for its direct consumption.

#### Summary

Closed-chamber experiments with rice seedlings attached to the seeds yield results which show that the dry weight of the young plants, particularly that of the roots, is increased by an increase of oxygen concentration up to as high as 50.1 per cent. oxygen both in darkness and in continuous illumination. Results of the solution culture experiment with the seedlings detached from the seeds indicate that the growth of the plants is unaffected by oxygen concentrations ranging from 0.2 to 20.8 per cent. oxygen prevalent in the environment of the root system in a wide variety of nutrient solutions.

In stagnant, nitrate-containing cultures, the rice plant within one week develops chlorosis of the central leaves; a symptom typical of iron deficiency. Tip-burn of lower leaves suddenly appears after two weeks in an ammonium-containing solution. Both of these symptoms are avoided by the addition of sodium thioglycollate at a concentration of 250 p.p.m. to the



respective solutions. Nitrate-supplied plants give much better root growth than the ammonium-supplied plants.

A possibility of upland cultivation of paddy rice is indicated.

Acknowledgement is made to the Department of Botany and Plant Pathology of Cornell University for the facilities so generously placed at the writer's disposal.

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# FACTORS INFLUENCING THE VOLATILE OIL CONTENT OF THE PEEL OF IMMATURE AND MATURE ORANGES<sup>1</sup>

E. T. BARTHOLOMEW AND WALTON B. SINCLAIR

(WITH FIVE FIGURES)

## Introduction

A study was made recently in this laboratory on some possible causes of breakdown in the peel of Washington Navel oranges. One approach to this problem was the determination and evaluation of the relative quantities of volatile oil in the peel of affected and unaffected fruits (2). It was found early in this investigation that more information was needed than had been published on the factors influencing the oil content of orange peel. Experiments were planned, therefore, to determine the effect of age and size of fruit, and the effect of environment, on the relative amounts of oil in the peel of healthy Valencia and Washington Navel oranges.

The results of these studies are reported in the present paper. They are considered to be important not only because of their bearing on the commercial production of citrus oils, but also because of the relation of the oil to certain discolorations and pittings of the peel when the oil is liberated from the glands by excessive turgidity or by fungus, insect, or mechanical wounds.

Hood (7), in Florida in 1916, and WILSON and YOUNG (13), in California in 1917, appear to have been the first and only ones in the United States to have made quantitative studies on the volatile oil in the peel of citrus fruits, except possibly on a commercial basis. Comparatively recently, determinations of the volatile oil in citrus peel have been made in other countries by DE VILLIERS (5), TANCHICO and WEST (11), BRAVERMAN and MONSELISE (4), SAMISCH (9), and FELIÚ (6). The sizes of the samples and the methods used in the last three studies appear to warrant conclusions only as to general trends.

## Materials and methods

To determine the effect of size and age of fruit on the oil content of the peel, Washington Navel and Valencia oranges were taken at approximately monthly intervals from plots of 50 trees each, selected for this study. The test period extended from August 22, 1944, to March 13, 1945, for Navels, and from August 30, 1944, to May 29, 1945, for Valencias. For each sample, 4 medium-sized fruits were picked at intervals around the circumference of each of the 50 trees in a plot, and the 200 fruits were divided at random into duplicate lots of 100 fruits. The influence of maturity on the yield of oil was further studied by determining the oil content of the peel of Valencias that were several months past their initial stage of commercial maturity (the stage at which the ratio of soluble solids to acids is 8:1).

<sup>1</sup> Paper no. 538, University of California Citrus Experiment Station, Riverside, California.

The effect of environment on the oil content of the peel of citrus fruits was determined by obtaining, from packing houses in various districts of southern California, mature Navels and Valencias from different groves. The Navels were obtained during March and April, the Valencias during July and August. Each sample consisted of 50 fruits taken at random from 10 or more field boxes on the receiving floor of the packing house. The Navels in the districts from which the test fruits were chosen reach commercial maturity from late December to the middle of January, the Valencias from early April to early May.

Different investigators have obtained the oil from citrus peel by various methods: namely, by cutting the whole fruit, the peel, or only the outer portion of the peel into pieces and then grinding and distilling; by puncturing the surface of the peel and taking up the oil in a solvent; or by simple hand or mechanical pressure. There are several objections to quantitative determination of the oil content of citrus peel by these methods: (a) when the whole fruit is used, under the usual laboratory conditions, the number of fruits tested is likely to be too small to give a representative yield of oil; (b) when the peel is stripped from the fruit, and especially when only the outer surface of the peel is cut off in thin strips, there is necessarily considerable loss of oil; (c) when a solvent is used it is very difficult to exclude the water in order to make an accurate quantitative reading; and (d) it is readily realized, of course, that quantitative determinations cannot be made by the pressure method of removing the oil from the peel.

These difficulties were avoided in the following manner. Holes about  $1\frac{1}{4}$  inches in diameter were punched in corrugated cardboard attached to a wooden frame. The fruits were placed over these holes in the desired position—stem end up, styler end up, or horizontally. A limited area on the upper portion of each fruit was then coated with dilute shellac and quickly dried with a fan. Either one or two disks (one from each fruit of the 100-fruit samples and two from each fruit of the 50-fruit samples) were cut in the shellacked area with a cork borer having an inside diameter of 15.7 mm. A sharp knife was next used to cut under the disks tangentially to the surface of the fruit. In this manner the disks of peel were removed with a minimum loss of oil.

The distribution of the oil in the peel was determined quantitatively by taking disks of peel from the stem and styler (proximal and distal) ends or from the equatorial regions of the fruits. Unless otherwise stated, the disks were from the equatorial regions.

If the fruits were green, they were allowed to remain in the laboratory overnight or longer to lose some of their turgidity before removal of the disks. The 100 disks used for each determination, with about 300 ml. of distilled water, were placed in a Waring Blendor and ground very fine. The grinding time for disks from green fruits was 8 to 10 minutes and for disks from mature fruits, 5 to 6 minutes. After grinding, the material was transferred to a 2-liter flask which was then connected to the apparatus

designed by BARTHOLOMEW and SINCLAIR (3) for the distillation and determination of citrus oils. With disks of moderate size, the sample of peel from 50 or 100 fruits is representative without being too bulky to grind and distill.

The surface areas of the fruits were determined by measuring their major and minor axes and then using tables (compiled by the authors but unpublished<sup>2</sup>) for computing the surface areas of prolate and oblate spheroids from such measurements. The area per disk and the weight of each lot of fruit were determined also. With these data and the use of a specific gravity factor of 0.8440 in all weight computations (8), it was a matter of simple calculation to determine the yield of oil per given unit of surface area, the mean yield per fruit, and the yield per ton of fruit. All values reported are based on yield per unit of surface area because the oil glands are all located in the surface layers of the peel, the flavedo. Tests showed that no oil could be obtained from the white portion of the peel, the albedo.

The temperature of the oil at each reading was recorded, but since the differences were small, corrections were not made on the values presented. Chemical and physical constants of the oil were not determined. POORE (8) has already determined these characteristics for California lemon and orange oils.

### Results

#### CHANGES IN THE VOLATILE OIL CONTENT OF VALENCIA ORANGE PEEL DURING FRUIT DEVELOPMENT AND AFTER MATURITY

This study involved the quantitative determination of the volatile oil in the peel of Valencia oranges picked at approximately monthly intervals from the time they were very green (August 30, 1944) until about two months after they had reached commercial maturity (May 29, 1945). During this period the mean surface area per fruit increased from 53.8 cm.<sup>2</sup> to 122.7 cm.<sup>2</sup> (table I). It is of interest to note further that, regardless of the change in maturity and the increase in size of the fruits, there was little change in mean yield of oil per unit area of peel. On the other hand, the yield of oil per fruit, on the basis of mean surface area, increased from 0.42 ml. to 1.09 ml. These results show that the yield of oil in the peel, as has been reported by DE VILLIERS (5), is correlated with the surface area of the fruit.

Since the oil is contained in the glands of the peel, it is important to know the relation between the oil-gland density and the yield of oil. TURRELL and KLOTZ (12) have shown that the mean density of oil glands per square millimeter of peel of navel orange fruits is 2.49 for small fruits, 2.33 for medium-sized fruits, and 2.17 for large fruits. (The mean equatorial diameters of the fruits tested were as follows: small, 6.17 cm.; medium, 7.13 cm.; and large, 8.09 cm.). The small fruits thus had 14.8 per cent. more oil glands per unit area than the large fruits. On this basis, it is evident from

<sup>2</sup> DR. F. M. TURRELL of this laboratory is publishing, in book form, a much more complete set of tables on surfaces and volumes of spheres and of prolate and oblate spheroids (University of California Press).



TABLE I

CHANGES IN THE VOLATILE OIL CONTENT OF VALENCIA ORANGE PEEL DURING GROWTH OF FRUIT

SAM- PLE	DATE FRUIT PICKED, 1944-45	MEAN SURFACE AREA PER FRUIT	MOISTURE IN PEEL (FRESH-WEIGHT BASIS)			MEAN YIELD OF OIL IN PEEL			
						PER 100 CM. <sup>2</sup>			PER FRUIT
			STEM END	STYLAR END	MEAN	STEM END	STYLAR END	MEAN	
		cm. <sup>2</sup>	%	%	%	ml.	ml.	ml.	ml.
1	Aug. 30	53.8	Not determined			0.76	0.82	0.79	0.42
2	Oct. 5	74.9	74.5	72.4	73.5	0.76	0.82	0.79	0.59
3	Nov. 16	95.2	76.3	73.8	75.1	0.75	0.83	0.79	0.75
4	Dec. 14	103.2	74.3	71.5	72.9	0.70	0.76	0.73	0.76
5	Jan. 15	115.6	74.9	71.4	73.2	0.70	0.79	0.75	0.86
6	Feb. 12	116.6	75.0	69.4	72.2	0.73	0.86	0.80	0.93
7	Mar. 19	117.7	75.6	70.4	73.0	0.74	0.81	0.78	0.91
8*	May 29	122.7	76.6	69.0	72.8	0.83	0.94	0.89	1.09

\* Sample 8 was fully mature fruit having a ratio of soluble solids to acids greatly exceeding 8:1, the minimum requirement for fruit marketed in California. The small surface area is indicative of the small size of the fruit of the 1945 crop.

the data in table I that the glands in the peel of the large Valencia fruits must have increased in size, or at least contained a greater quantity of oil

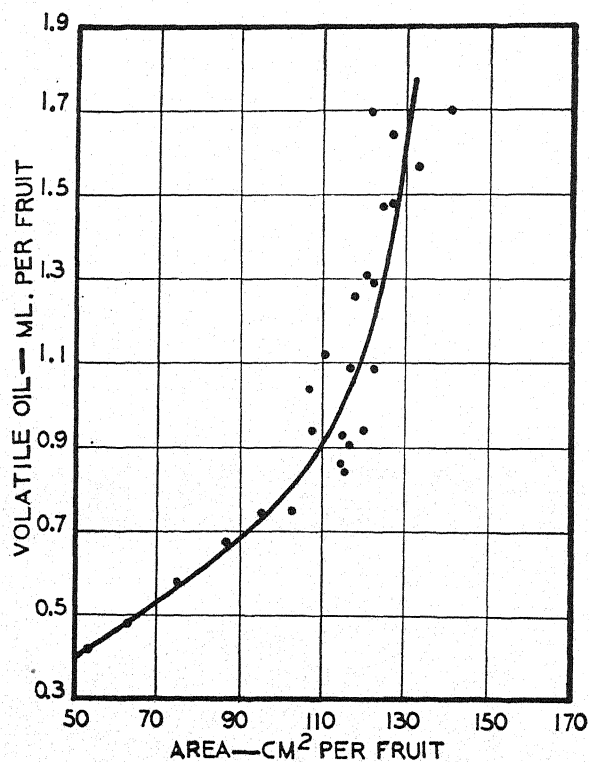


FIG. 1. Volatile oil content of the peel of Valencia oranges, in relation to fruit size.

per gland than those in the small fruits. This is shown by the fact that the mean increase in yield of oil per fruit, between August 30, 1944, and May 29, 1945, was 388 per cent., whereas the mean increase in surface area per fruit during this period was only 280 per cent.

Figure 1, as well as table I, shows that up to the time the Valencia fruit is mature and nearly full size, the yield of oil is directly correlated with the surface area of the fruit. Figure 1 shows, also, that after the fruit has reached this stage of development, the oil content of the peel is governed more by climatic or physiological conditions than by fruit size. This is shown by the sharp rise in the curve and by the scattering of the points after the fruits had attained a surface area of about 120 cm.<sup>2</sup> After the fruits had reached this size, the yields from some lots were almost twice as much as those from other lots, and yet there was very little change in fruit size. The point at which the curve will turn upward apparently depends on the size of the mature fruits, and varies from year to year. The season of 1944-45 happened to be one of small sizes.

TABLE II

VOLATILE OIL IN THE PEEL OF MATURE VALENCIA ORANGE FRUITS FROM DIFFERENT DISTRICTS IN SOUTHERN CALIFORNIA

NUMBER OF SAMPLES TESTED*	DATE FRUIT PICKED, 1945	MEAN DIAMETER OF FRUITS OF EACH LOT	MEAN YIELD OF OIL IN PEEL	
			PER 100 CM. <sup>2</sup>	PER TON OF FRUIT
		cm.	ml.	lb.
INLAND DISTRICT				
1	July 11	6.2	1.29	20.2
2	July 13	6.3	1.29	20.7
2	July 17	5.9	0.97	16.2
4	July 18	6.2	1.06	16.9
7	July 23	6.1	1.10	17.8
Mean .....	.....	.....	1.14	18.4
Av. dev. ....	.....	.....	± 0.12	± 1.68
INTERMEDIATE DISTRICT				
7	July 25	6.0	0.89	15.2
6	Aug. 3	6.0	0.89	14.7
4	Aug. 7	6.2	0.82	13.6
4	Aug. 10	5.9	0.89	14.9
Mean .....	.....	.....	0.87	14.6
Av. dev. ....	.....	.....	± 0.03	± 0.50
COASTAL DISTRICT				
10	Aug. 11	6.2	0.82	14.1
5	Aug. 21	6.1	0.73	12.3
5	Aug. 23	6.2	0.91	14.8
5	Aug. 28	6.1	0.76	12.9
4	Aug. 30	6.1	0.73	12.4
Mean .....	.....	.....	0.79	13.3
Av. dev. ....	.....	.....	± 0.06	± 0.92

\* Each sample came from a different grove—a total of 66 groves.

EFFECT OF ENVIRONMENT ON THE VOLATILE OIL CONTENT OF THE PEEL  
OF MATURE VALENCIA AND NAVEL ORANGES

In order to determine whether there are differences in the oil content of the peel of oranges grown in different districts, sample lots of Valencias and Navels were obtained as previously described (see "Materials and methods"), and the oil content was determined.

The results of the study on Valencias are shown in table II. The fruits were 4 to 5 months past the initial stage of commercial maturity, and all had approximately the same mean equatorial diameter. The most striking feature of the data (table II) is that the oil content of the peel was highest in inland fruits and progressively lower in those grown nearer the coast. The mean oil content of the peel of the inland fruits, on a tonnage basis, was 38.3 per cent. greater than that of the coastal fruits.

A similar study was made on Navel oranges in inland and intermediate districts only (fig. 2). The average yield of oil per fruit is plotted against

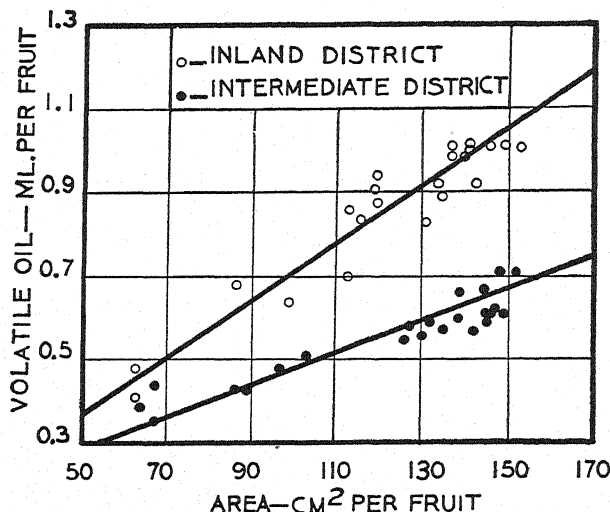


FIG. 2. Changes in the volatile oil content of the peel of Navel oranges from two different districts in southern California with increase in fruit size.

the average area per fruit in each of the two districts. The yields of oil from the fruits in the inland district were greater than those from the fruits in the intermediate district. Since there is no sharp upward trend in the curves in figure 2, it would appear that there is not the noticeable increase in oil in Navels that there is in Valencias (fig. 1) after they have reached maximum size. This may be due to the fact that Navels mature and are picked during the winter months when food manufacture is at a minimum.

The results portrayed in figure 2 show also that, in general, a straight-line relation existed between the increase in yield of oil per fruit and the average area per fruit. There was no tendency for the yield of oil to decrease, although the last values were determined after the fruits were 3 to 4

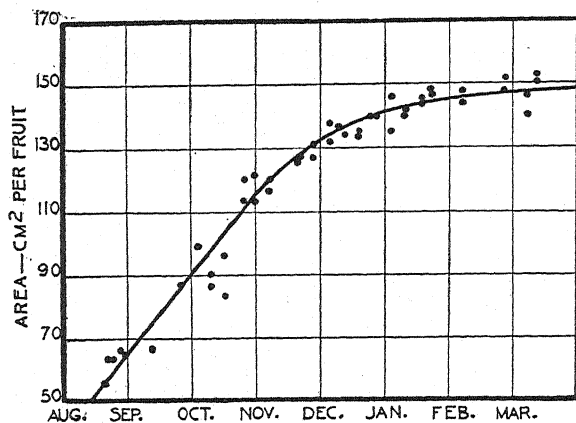


FIG. 3. Changes in mean surface area per fruit (Navel oranges) as the season advanced.

months past their initial stage of commercial maturity. At the time the final sample was taken the fruits had reached their maximum size (fig. 3). The values on the curve represent fruits from both the inland and the intermediate districts. For any given month the fruits from both districts were approximately the same size.

The size of the fruit in a sample, taken from either the inland or intermediate districts, apparently did not affect the amount of oil obtained per 100 cm.<sup>2</sup> of peel (fig. 4). Throughout the experimental period for both districts (September to February, inclusive), fruits from the inland district contained nearly twice as much oil per 100 cm.<sup>2</sup> of peel as those from the intermediate district.

Further evidence that the amount of oil in the peel of Valencia oranges in the inland districts does not decrease after they have become mature is

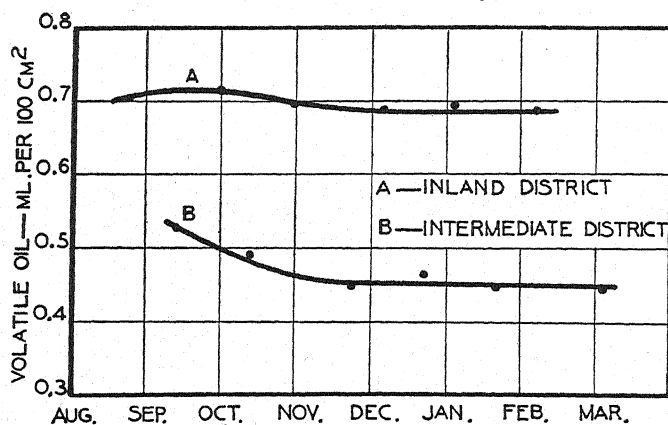


FIG. 4. Volatile oil content of peel of Navel oranges of different ages, from two different districts in southern California. Within a given district, the oil content per 100 cm.<sup>2</sup> of fruit surface showed comparatively little change. Between the two districts, the differences are obvious.

TABLE III

VOLATILE OIL IN PEEL OF OVERMATURE VALENCIA ORANGES FROM THE INLAND DISTRICT OF SOUTHERN CALIFORNIA

DATE FRUIT PICKED, 1945	MEAN SURFACE AREA PER FRUIT	MEAN YIELD OF OIL IN PEEL	
		PER 100 CM. <sup>2</sup>	PER TON OF FRUIT
	cm. <sup>2</sup>	ml.	lb.
July 23	126.9	1.17	18.7
Sept. 4	141.8	1.20	18.3
Oct. 9	133.1	1.18	18.5
Nov. 6	131.3	1.21	19.3

shown by the data in table III. The first lot of fruit was tested about 4 months and the last lot about 8 months after the fruit had become commercially mature. The values were approximately the same for the first three tests; the last test indicates a slight but probably insignificant increase in yield. These results agree with those of BRAVERMAN and MONSELISE (4), but do not agree with those of HOOD (7), SAMISCH (9), and FELIÚ (6), who found that the yield of oil began to decrease after the fruits had become mature.

EFFECT OF FRUIT SIZE ON THE VOLATILE OIL CONTENT OF THE PEEL  
OF MATURE VALENCIA ORANGES

The relative yields of oil from the peel of large and small mature Valencia oranges are shown in table IV. The samples of large and small fruits were

TABLE IV

RELATIVE AMOUNTS OF VOLATILE OIL IN THE PEEL OF LARGE AND SMALL, MATURE VALENCIA ORANGES FROM DIFFERENT DISTRICTS\*

SAMPLE	LARGE FRUITS			SMALL FRUITS		
	MEAN SURFACE AREA PER FRUIT	MEAN YIELD OF OIL IN PEEL		MEAN SURFACE AREA PER FRUIT	MEAN YIELD OF OIL IN PEEL	
		PER 100 CM. <sup>2</sup>	PER TON OF FRUIT		PER 100 CM. <sup>2</sup>	PER TON OF FRUIT
	cm. <sup>2</sup>	ml.	lb.	cm. <sup>2</sup>	ml.	lb.
1	151.8	1.19	17.6	102.0	1.15	19.8
2	149.9	1.17	17.6	103.9	1.14	20.0
3	143.9	0.92	14.3	97.7	0.93	17.3
4	150.2	0.89	13.6	102.3	0.84	15.5
5	148.0	0.77	12.1	104.6	0.77	13.6
6	148.4	0.97	14.6	96.0	0.90	16.0
7	151.0	0.88	13.5	110.5	0.87	15.5
8	173.8	1.10	15.8	105.4	1.00	17.0
9	154.8	0.75	11.2	103.2	0.75	13.1
10	156.8	0.69	10.3	104.8	0.65	11.5
Mean .....	152.9	0.93	14.1	103.0	0.90	15.9
Av. dev. ....	.....	± 0.14	± 1.9	.....	± 0.12	± 2.1

\* The mean equatorial diameter of the large fruits was  $6.9 \pm 0.13$  cm. (approx.  $2\frac{3}{4}$  in.), and of the small fruits,  $5.7 \pm 0.08$  cm. (approx.  $2\frac{1}{4}$  in.). Oranges of these sizes in California are classed as 200's and 324's, respectively.

taken at random from the same boxes on the receiving floor of packing houses in different districts. The mean surface areas of the fruits in the different lots are shown in the table.

The yield of oil per 100 cm.<sup>2</sup> of fruit surface was practically the same for large and small fruits. The yield of oil per ton of fruit, however, was noticeably larger for the small fruits than for the large ones. This was to be expected because, as already shown, the yield of oil is a function of the surface area of the fruit. That it is a function of the surface area is further demonstrated in figure 5, which shows the yields of oil in pounds per ton of

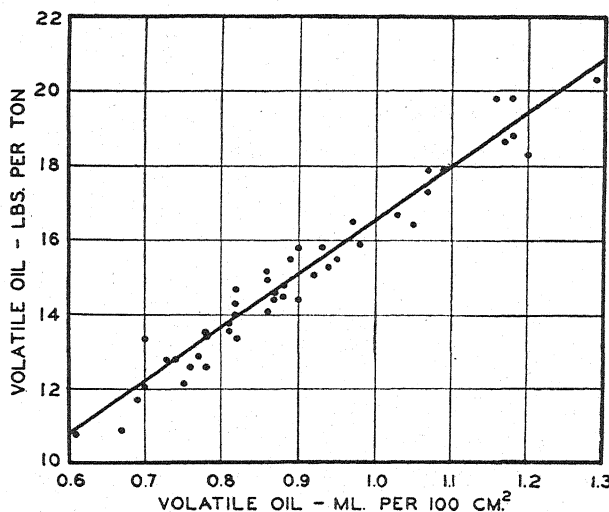


FIG. 5. Yield of oil, in pounds per ton of mature Valencia oranges, correlated with the yield in milliliters per 100 cm.<sup>2</sup> of surface area.

mature Valencias, computed from the yields of oil in milliliters per 100 cm.<sup>2</sup> of surface area.

#### VOLATILE OIL CONTENT OF THE PEEL OF THE STEM AND STYLAR ENDS OF VALENCIA ORANGES

A study of the relative amounts of volatile oil in the peel of the stem and stylar ends of the fruits was made because various portions of the peel sometimes react differently under pathological conditions. The study was made, also, to determine whether various portions of the peel contain different amounts of oil, just as various portions of the pulp contain different amounts of soluble solids. [Refer to "Literature Cited" in report on studies by BARTHOLOMEW and SINCLAIR (1).]

The results (table V) show that considerably more oil was obtained from the stylar end than from the stem end of the fruit. In samples 1 to 6, the disks of peel were taken about midway between the equator and the stem and stylar ends, respectively, of the fruits. In these samples the disks from the stylar end contained 11 per cent. more oil than those from the stem end.



(For similar results, see table I.) In samples 7 to 12, the disks were taken so that the nearest edge of the disk was approximately 7 mm. from the exact center of the stem or styler end, respectively, of the fruit. In this case the disks from the styler end contained 33 per cent. more oil than those from the stem end. These results indicate that the oil content of the peel of Valencia oranges increases progressively from the stem end to the styler end of the fruit. A similar condition was found with Navel oranges, although the differences were not so great and there were some exceptions.

### Discussion

The colorless oil obtained from the peel of the orange by distillation was taken as the total oil content. It is probable that small amounts of the constituents of the oil as it exists in the glands were oxidized during distillation. This, however, did not prevent relative values from being surprisingly constant. Most determinations were made in duplicate, and the variations in such determinations were very small, ranging from 0 to 0.052 ml. per 100 cm.<sup>2</sup> of peel.

The volatile oil is located in specialized receptacles (glands) in the surface layers of the peel, regardless of its thickness. Therefore, although data are presented which show the yield of oil per ton of fruit, it seemed that a more accurate method would be to express the yield as a function of the surface area of the peel. To express the yield of oil as a percentage of the wet or dry weight of the peel would not be satisfactory for interpreting physiological or chemical data, owing to the fact that the peel may be much thicker on some fruits than on others. It can be seen readily that two samples of peel of different thickness might have the same oil content per unit of surface area, but might yield different percentages of oil if calculated on the weight of the same area of peel. Had the yield of oil been expressed on a weight basis, the water content of the peel would have been a factor, but the yields in table I, which are based on surface area, indicate that there is no direct correlation between yield of oil and the amount of water in the peel.

The variations in comparable values in tables II, IV, and V, and the scattering of the points in figures 1, 2, 4, and 5, are due largely to climate, cultural methods, and other factors that prevailed in the districts and groves from which the fruit samples came. Similar results have been reported by HOOD (7), and by WILSON and YOUNG (13). To a much less degree the variations can be attributed also to errors in random sampling and in converting the yield values from comparatively small samples into tonnage values. The slight degree of variance between the latter values does not, however, prevent the reported results from being significant and of practical importance.

The finding that the quantity of oil in the peel of oranges is influenced by conditions existing in the district or grove in which the fruit is grown parallels earlier findings that the quantity of soluble solids in the juice is affected by the same factors (10).

The yield of oil from Valencias was much higher than that from Navels. The mean yield from 21 samples of Valencias from 21 groves in the inland district was 1.10 ml. per 100 cm.<sup>2</sup> of peel, whereas that from 30 similar samples of Navels was only 0.52 ml. per 100 cm.<sup>2</sup> of peel, a difference of 0.58 ml. A similar relation existed between the yields from fruit from the intermediate districts, the mean yield for Valencias being 0.88 ml. per 100 cm.<sup>2</sup> of peel, and for Navels, 0.43 ml. There was a greater difference between the yields for Valencias in the two districts (1.10 ml. and 0.88 ml.) than for the Navels (0.52 ml. and 0.43 ml.). In only one sample of Valencias was the yield lower than the highest yield from a sample of Navels. Both Valencias

TABLE V

RELATIVE AMOUNTS OF VOLATILE OIL IN PEEL OF STEM AND STYLAR ENDS  
OF MATURE VALENCIA ORANGES

SAMPLE	MEAN YIELD OF OIL IN PEEL OF			
	STEM END		STYLAR END	
	PER 100 CM. <sup>2</sup>	PER TON OF FRUIT*	PER 100 CM. <sup>2</sup>	PER TON OF FRUIT*
	<i>ml.</i>	<i>lb.</i>	<i>ml.</i>	<i>lb.</i>
1	1.23	19.3	1.35	21.2
2	1.34	21.7	1.43	23.2
3	0.99	16.0	1.07	17.3
4	1.01	15.8	1.09	17.0
5	1.00	16.5	1.17	19.3
6	0.73	12.4	0.89	15.1
Mean .....	1.05	17.0	1.17	18.9
Av. dev. ....	± 0.16	± 2.4	± 0.15	± 2.4
7	0.65	10.4	0.90	14.8
8	0.71	12.7	0.93	16.6
9	0.68	11.9	0.95	16.6
10	0.67	11.5	0.89	15.2
11	0.82	14.4	0.97	17.1
12	1.00	15.3	1.39	21.2
Mean .....	0.76	12.7	1.01	16.9
Av. dev. ....	± 0.11	± 1.4	± 0.13	± 1.5

\* Based on oil content of peel of stem and stylar ends, respectively.

and Navels were of similar maturity—about 3 to 4 months beyond the initial stage of commercial maturity.

Distribution studies of the oil glands in the peel of Valencia oranges were not made. The data presented indicate, however, that the distribution is similar to that of Navels (12). The larger yield of oil for Valencias indicates, also, that the oil glands per unit of surface area are more abundant, or larger, on the Valencias than on the Navels, or that the glands of the Navels contain more non-oil substances than those of the Valencias.

### Summary

Changes in the volatile oil content of the peel of Washington Navel and Valencia oranges with fruit development have been determined on fruit

samples picked from experimental plots at intervals during the season. Variations caused by environmental factors have been demonstrated by determining the volatile oils on mature fruit samples from groves in widely separated areas of southern California.

As Valencia oranges mature and increase in size, the yield of oil per unit area of fruit surface shows little change, but the yield based on the mean surface area per fruit increases tremendously. Up to the time the Valencia fruits are mature and nearly full size, the oil content is directly correlated with the surface area of the fruit, but after the fruits have reached this stage of development, the oil content is governed more by climatic and physiological conditions than by fruit size.

The oil content of the peel of both Navels and Valencias is highest in fruits from the inland district and progressively lower in those grown nearer the coast. Large fruits yield more oil than small fruits when the oil content is expressed as yield per surface area, but the reverse is true when the yields are expressed on a tonnage basis, because small fruits have more surface area per ton. Quantitative determinations indicate that the oil content of the peel increases progressively from the stem end to the stylar end of the fruit. The yield of oil per unit area (100 cm.<sup>2</sup>) of fruit surface is highly correlated with the yield of oil per ton of fruit. The yield of oil from Valencias is much greater than that from Navels.

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## THE EFFECT OF TOP ENVIRONMENT AND FLOWERING UPON TOP-ROOT RATIOS<sup>1</sup>

R. H. ROBERTS AND B. ESTHER STRUCKMEYER

WITH EIGHT FIGURES

Several papers and many references in other manuscripts have dealt with the relation of growth of the tops and growth of the roots of plants. These generally present data showing the effect of soil types, moisture or mineral elements upon top-root ratio (2, 4, 10). The idea is quite prevalent in some agricultural circles that the relative amount of roots on a plant is determined, if not controlled, by the soil. On the other hand, there are several other factors which can be shown to modify greatly the top-root ratio. Among these are variety, light intensity (10), photoperiod, partial defoliation, girdling, fruiting, and growth substances (14). For example, the amount as well as character of the root system of piece-root grafted apple trees varies with the variety of scion used (5, 9). The type or amount of reserves accumulated in perennial plants as the apple has marked effects upon the subsequent top-root ratio (fig. 1). It has been reported in the literature that plants grown in short days have relatively few roots (1, 11). This conclusion may be strengthened if the plants are not grown to maturity. Also, having few roots in short photoperiods is an effect which may be incidental to the fact that the plants under observation come to flower in short days. Plants which blossom in long photoperiods have been seen to have fewer roots in long photoperiods (6), indicating a correlation between flowering and limited root development. This was found to be the case by WITHROW, who used nitrogen supply as a variable with photoperiod (13). She states: "... plants, for the most part, were able to form proportionately larger tops as compared to roots under that photoperiod which brought about flowering as compared to that photoperiod under which the plants remained vegetative. Nitrogen supply did not usually alter the direction of this response, although under either photoperiod there were proportionately larger tops as compared to roots at high nitrogen levels than when the nitrogen supply is limited." Nitrogen is generally credited with increasing the top-root ratio (2, 4, 10).

The data presented in this paper were collected to measure to what extent the ratio of top to root is influenced by some factors in the environment of the top; also, to see if the relative amount of root formation is consistently related to the function of flowering and fruiting regardless of whether a plant has a greater ratio of roots in short photoperiods, long photoperiods, cool temperatures, warm temperatures or other specific cultural environment.

<sup>1</sup> Published with the approval of the Director of the Agricultural Experiment Station.

## Materials and methods

Twenty-nine species or varieties of plants were grown in some or all of the following six environments: long and short photoperiods in the greenhouse, at three minimum (night) temperatures: 55°, 65°, and 75° F. Plant-

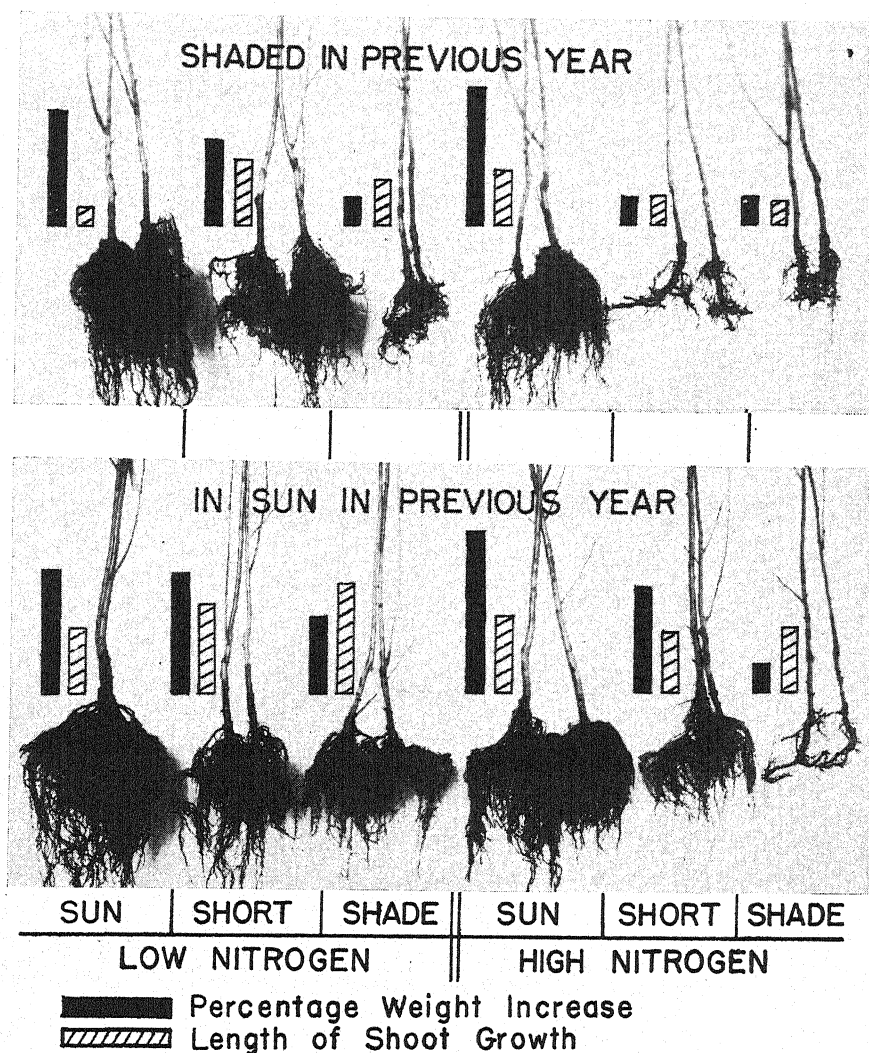


FIG. 1. Effect of light (sun, shade, and photoperiod) and nitrogen nutrient upon root development, weight increase, and shoot growth of Wealthy apple trees in pots. 1927. Upper row of trees were from a lot shaded the previous year when growing in the nursery; lower row from trees grown in full sunlight.

ings were made in the winter of 1940-41, and many were repeated in the following greenhouse season. The plants used for ratio determinations were chosen for their previously ascertained responses to photoperiod and



temperature (6, 7) to provide a wide range of top and root development as well as flowering.

Seedling plants were pricked off into flats and later transplanted to pots according to usual greenhouse procedure. Prior to potting and transfer to the experimental treatments the seedlings or cuttings of varieties which are responsive to photoperiod were kept in an environment unfavorable to blossoming.

Plants of many of the lots were sampled at intervals to secure data for different stages of development of the plants. As a rule, three plants of a lot were taken for a single sample. This number was varied somewhat depending upon the size or bulk of the specimens. The roots were carefully washed free of soil and separated from the tops; or, in cases where plants were started as cuttings, both tops and roots were separated from the original portion, the latter being discarded.

The samples were brought to dryness at 80° to 85° C. The ratios of top-to-root were calculated on a dry weight basis.

### Results

The influence of temperature, photoperiod, and flowering upon the top-root ratio of most of the varieties of plants studied is shown by table I.

Before summarizing the effects of photoperiod, temperature and fruiting upon the top-root ratio, some observations will be made upon the history, special cultural conditions or reactions of some of the lots listed in table I.

Lot 1, Sunflower. During the latter part of the growing period the minimum night temperature of 55° F. in the cool hours could not always be maintained. The effect of the treatment during the early phases of growth did, however, produce plants very much unlike those in the warmer houses (fig. 2).

Lot 2, Alfalfa. The plants made responses typical of alfalfa; that is, plants placed in a warm location remain weakly vegetative after early limited flowering; the medium temperature, long-day plants blossom, but these generally abort (4); and the cool, long photoperiod plants fruit well although slowly. The cool, short-day plants grow slowly and remain strongly vegetative (fig. 3). An interesting feature of this graph is the change in top-root ratio as the flowering state is passed by the warm plants and as the cool, long photoperiod plants come to flower.

Lots 3, 4, Spinach. Spinach plants "go to seed" very quickly in warm and medium temperatures, especially in long but to some degree also in short photoperiods. Plants remain in a rosette stage of growth for months when grown in cool, short photoperiods; the cool, long-photoperiod plants produce seed stalks at a young age, and these often grow to several feet in height. The top-root ratios of the cool temperature plants seeded on November 29 are shown by figure 4. Again, the seasonal change in top-root ratio as flowering occurs can be noted and also the extremely high top-root ratio of the fruiting long photoperiod plants.

TABLE I  
REPRODUCTIVE STATE AND RATIO OF TOP TO ROOT, DRY WEIGHT

DATE TREATMENT STARTED	DATE SAMPLE TAKEN	PLANT	LOT NO.	75° F.		65° F.		55° F.	
				LONG DAY	SHORT DAY	LONG DAY	SHORT DAY	LONG DAY	SHORT DAY
LONG-DAY TYPES									
3-10 <sup>b</sup>	5-27	<i>Helianthus cucumerifolius</i> , var. Orion	1	F*7.0	NF4.9	F10.9	NF4.0	F8.8	NF3.7
1-18 <sup>c</sup>	4-10	<i>Medicago sativa</i> , strains H-822-1	2	F-NF4.8	F-NF5.7	F3.8	NF2.9	B7.5	NF1.9
11-29 <sup>d</sup>	4-1	<i>Spinacia oleracea</i> , fig. 4	3					F34.2	NF6.6
12-4 <sup>d</sup>	2-2	"	4	F19.1	NF7.6	F14.9	NF6.8	F3.9	NF2.8
1-2 <sup>e</sup>	4-2	<i>Agropyrum repens</i> (Quack grass)	5	F12.5	F-NF6.9	F9.8	NF6.7	F10.4	NF3.6
12-12 <sup>f</sup>	4-4	<i>Melilotus alba</i> (Sweet clover)	6	F14.5	NF10.3	F11.8	NF6.8	T <sup>g</sup> 00	Cool
11-29	3-21	<i>Mathiola incana</i> , var. Christmas Pink	7	NF7.8	NF7.2	NF6.8	NF6.9	B5.7	NF4.6
1-19	5-26	" (Stock)	8	NF7.5	NF13.0	B16.5	NF8.5	F13.9	B7.6
11-28	4-10	<i>Poa pratensis</i> , strain 5 (Blue grass)	9	NF7.5	NF8.2	NF7.4	NF6.0	B2.7	NF2.6
Same	Same	" " 12	10	NF5.7	NF6.4	NF6.4	NF4.0	NF3.1	NF2.4
1-10 <sup>g</sup>	3-17	<i>Brassica oleracea</i> , var. capitata	11	NF14.2	NF11.1	NF14.7	NF11.0	NF8.4	NF4.3
SHORT-DAY TYPES									
1-14 <sup>h</sup>	4-18	<i>Cineraria cruenta</i> , fig. 5	12	Dead	F14.2	NF3.4	F8.6	NF0.6	F2.1
12-21	3-3	<i>Panicum miliaceum</i> , var. German	13	NF5.4	F17.2	NF6.6	F12.2	NF3.2	B4.9
3-13 <sup>i</sup>	6-4	<i>Glycine Max</i> , var. Biloxi (Soybean)	14			NF5.6	F8.2		
4-22 <sup>k</sup>	6-3	<i>Xanthium echinatum</i> (cocklebur)	15	NF5.7	F17.2				
1-1	1-8	<i>Salvia splendens</i> , var. Harbinger	16	NF3.6	F12.7				
4-22 <sup>m</sup>	5-27	<i>Cosmos sulphureus</i> , var. Klondyke	17			NF5.5	B7.4		
11-24 <sup>n</sup>	1-20	<i>Datura stramonium</i> (Jimsonweed)	18	NF3.8	F7.2	NF4.4	F6.2	B4.8	

<sup>a</sup> F, flowering or fruiting; B, budding; NF, nonflowering; F-NF, regenerating vegetatively after blossoming; see fig. 3.

<sup>b</sup> Seed sown 2-11.

<sup>c</sup> Rooted cuttings of a clone having top growth of three to five weeks and a top root ratio of 4.1.

<sup>d</sup> Seeding date.

<sup>e</sup> Planting date of rhizomes from field plants.

<sup>f</sup> Planting date of crowns from field plants.

<sup>g</sup> Plants of transplant size for field setting.

<sup>h</sup> Seedlings with 5-6 leaves; the plants in warm, long days died.

TABLE I—(Continued)  
REPRODUCTIVE STATE AND RATIO OF TOP TO ROOT, DRY WEIGHT

DATE TREATMENT STARTED	DATE SAMPLE TAKEN	PLANT	LOT NO.	75° F.		65° F.		55° F.	
				LONG DAY	SHORT DAY	LONG DAY	SHORT DAY	LONG DAY	SHORT DAY
INTERMEDIATE TYPES <sup>n</sup>									
11- 8	12-23	<i>Fagopyrum esculentum</i> , var. Jap (Buckwheat)	19	F12.3	F13.1	F12.8	F8.9	F9.8	NF7.6
11-10	12-28	“	20	F12.4	F16.7	F15.4	F12.5	B10.5	NF8.6
11-22	2-18	<i>Nicandra physalodes</i>	21	F16.5	F24.9	F19.2	F16.7	F5.1	F6.8
1-20 <sup>o</sup>	5-20	<i>Antirrhinum majus</i> (Snapdragon)	22	NF9.3	NF14.7	NF12.4	NF10.9	F7.6	B5.9
11-12 <sup>o</sup>	5-26	<i>Pelargonium zonale</i> (Geranium)	23	NF6.1	B9.1	NF5.1	NF4.3	F10.3	F12.8
11- 5 <sup>o</sup>	5-26	<i>Phlox paniculata</i> , var. Miss Lingard	24	NF0.9	Dead	NF1.0	NF1.7	F2.3	B2.1
11-11 <sup>p</sup>	2-13	<i>Zea mays</i> , var. Wis. Golden Glow	25	F9.5	F9.7	NF4.8	F6.2		
2-17 <sup>d</sup>	4-12	<i>Pisum sativum</i> , var. Alaska	26			F13.7	F11.7	B8.0	B8.8
2-28 <sup>d</sup>	4-15	“	27			F11.2	F7.8	B5.3	B6.1
4-17 <sup>d</sup>	5-22	“	28	NF15.7 <sup>q</sup>	NF6.0	NF5.5	NF6.1		
3-20 <sup>d</sup>	4-15	“ , var. Prince of Wales	29	NF3.4	NF5.3	NF3.2	NF2.5	NF2.4	NF1.9
4-18 <sup>d</sup>	5-22	“	30	NF5.3	NF5.4	NF3.0	NF3.1		
1- 4 <sup>r</sup>	5-28	<i>Tradescantia</i> sp. (Wandering Jew)	31	B21.9	NF9.9	NF8.6	NF6.7		
11-19 <sup>d</sup>	12-31	<i>Phaseolus vulgaris</i> , var. B.S. Green Pod	32			F6.9	F7.5		
1-24	1-24	<i>Solanum nigrum</i> (Nightshade)	33			F4.9	B3.0		
3-18 <sup>o</sup>	4- 9	<i>Ipomoea batatas</i> (sweet potato)	34	NF6.0	NF4.6	NF3.4			
		Same, with tubers added to roots	35	3.2	4.6	1.9			
		<i>Tropaeolum majus</i> (Nasturtium)	36			F24.1			

<sup>i</sup> Seed planted 11-20.

<sup>j</sup> Seeded 1-10.

<sup>k</sup> Seeded 3-13.

<sup>l</sup> Older plants in blossom since November.

<sup>m</sup> Seeded 12-13.

<sup>n</sup> Plants which are not specific in their response to photoperiod or to temperature.

<sup>o</sup> Rooted cuttings.

<sup>p</sup> Seeded 11-1.

<sup>q</sup> Too warm to blossom normally.

<sup>r</sup> Cuttings of large, green-leaved variety.

<sup>s</sup> Older blossoming plants in good vigor.

or cool temperature. They may blossom more quickly either in warm, medium,

Lot 12, Cineraria. The high top-root ratio of this short-day plant, as it came to flower (fig. 5), is in contrast to the ratio situation in long-day plants; compare with figures 3 and 4.

Lot 13, Millet. The appearance of the plants at sampling time is shown

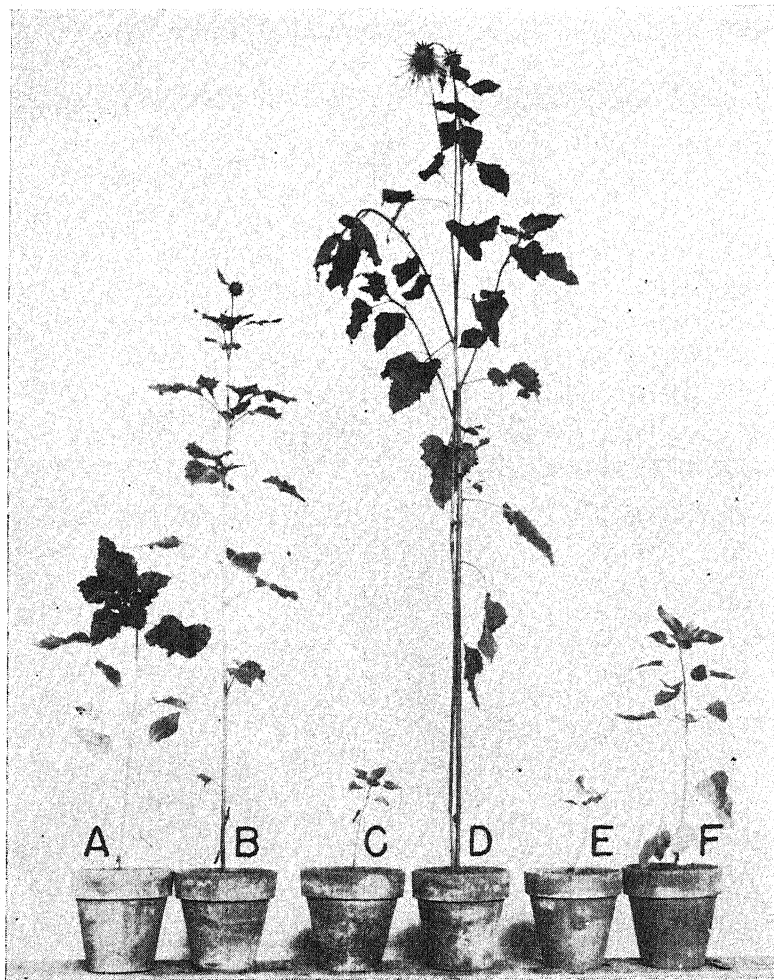


FIG. 2. *Helianthus cucumerifolius*, var. Orion. A, cool, short photoperiod; B, cool, long photoperiod; C, medium, short photoperiod; D, medium, long photoperiod; E, warm, short photoperiod; F, warm, long photoperiod, plants with poor flower development.

by figure 6. The high top-root ratio in short photoperiods is typical of short-day plants.

Lot 16, *Salvia*. Old plants were heavily top and root pruned January 8. The ratio of new growth of top and roots on March 18 was 2.4 for vegetative plants in long photoperiods and 4.0 for flowering plants in short photoperiods at a warm temperature.

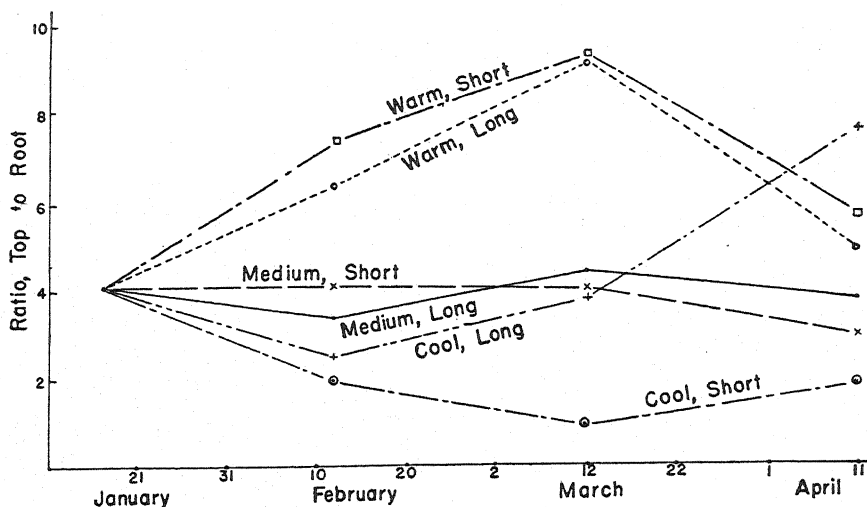


FIG. 3. Top-root ratio of alfalfa, strain H-822-1. Warm temperature plants blossom early and become vegetative later. Cool, long-day plants come to flower after an early vegetative period.

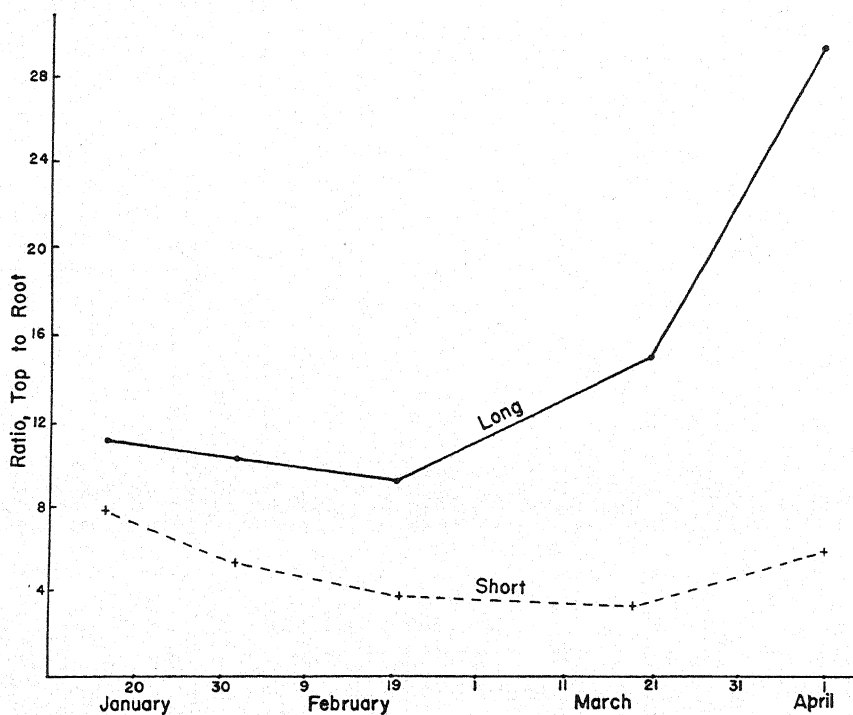


FIG. 4. Top-root ratio of spinach plants in a cool temperature. There is a marked increase of top in relation to the root as the long-photoperiod plants flower and fruit. The plants in short photoperiods remain vegetative.

Lots 19, 20, Buckwheat. This is the first indeterminate plant reported on. It is also a plant which forms blossoms when very young, and in large numbers. As would be expected if flowering plants have a limited root development, the top-root ratio is very high.

Lot 21, "Apple of Peru." This indeterminate plant might be expected to be valuable in determining the influence of temperature upon top-root ratio as it blossoms in both long and short photoperiods and in each temperature being used. Its value for this purpose is questionable until more is known of the effect of slower blossoming in the cool temperature upon top-root ratio.

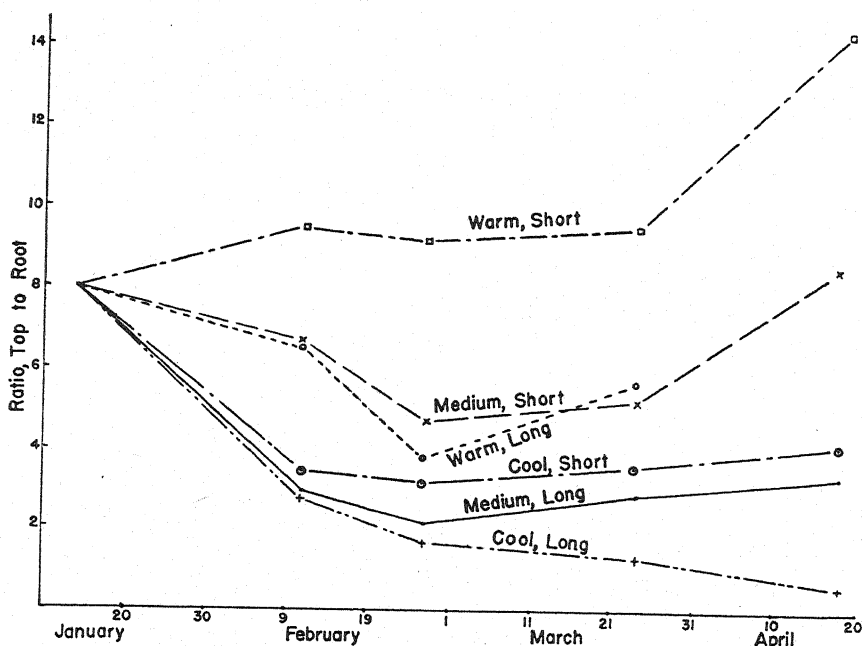


FIG. 5. Top-root ratio of *Cineraria cruenta*. The blossoming plants in short photoperiods had uniformly fewer roots in relation to the top than the non-blossoming plants in long days at each temperature.

Lot 25, Corn. This plant is sometimes considered as being a short-day species. The Mexican varieties are short-day types, but those grown in northern latitudes blossom earlier in short photoperiods only at cool temperatures for the species, as 60–65° F. In a warm environment of 75° F. varieties such as Wisconsin Golden Glow show little response to photoperiod so this plant should be classed as an indeterminate type. On March 14 the medium temperature plants in long and short photoperiods were both in tassel and had top-root ratios of 8.6 and 9.5, respectively.

Lots 26–30, Peas. Alaska peas need to be planted early for successful cropping. Later maturing sorts as Prince of Wales can be planted later with equal success. Early peas need cooler weather conditions. Possibly



this varietal difference may be due to the rooting habits. The early flowering sort, Alaska, had a much larger top-root ratio especially in the warmer temperature where fruits were produced early.

Hemp. The data on top-root ratio for *Cannabis sativa* var. Femarrington was not included in table I because of the lack of columns for male and female plants. This is a short-day plant at the three temperatures used, although some blossoming occurs on the older plants in long days at high temperatures. The particular item of interest in its response is the mark-

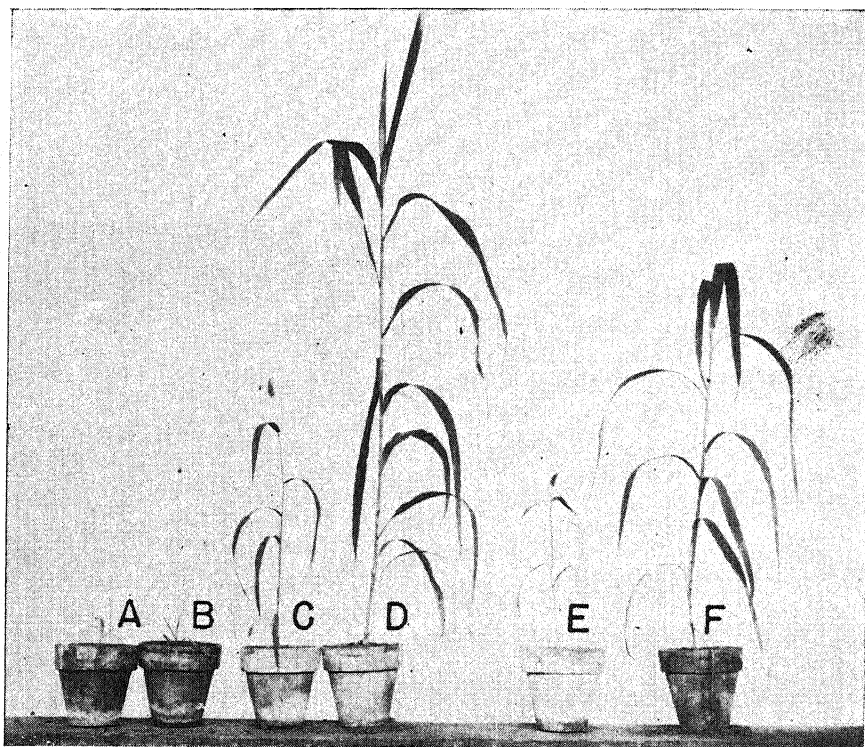


FIG. 6. German millet. A, cool, short photoperiod; B, cool, long photoperiod; C, medium, short photoperiod; D, medium, long photoperiod; E, warm, short photoperiod; F, warm, long photoperiod.

edly limited rooting of the male plants (fig. 7, D). (This may be a leaf relationship as the female plants have persisting green leaves and a greater leaf area.) The top-root ratios March 27 are shown with figure 7 for plants from seed planted November 28 and given environmental treatments beginning January 24.

#### EFFECT OF PHOTOPERIOD UPON TOP-ROOT RATIO

An examination of table I shows that the long-day plants (lots 1-11) have a consistently higher top-root ratio in long photoperiods, that is, at a given temperature. The short-day species (lots 12-18) have just as con-

sistently a higher top-root ratio in short photoperiods at the same temperature. In the case of indeterminate lots (lots 19-36) there is no pattern of photoperiod effect upon top-root ratio.

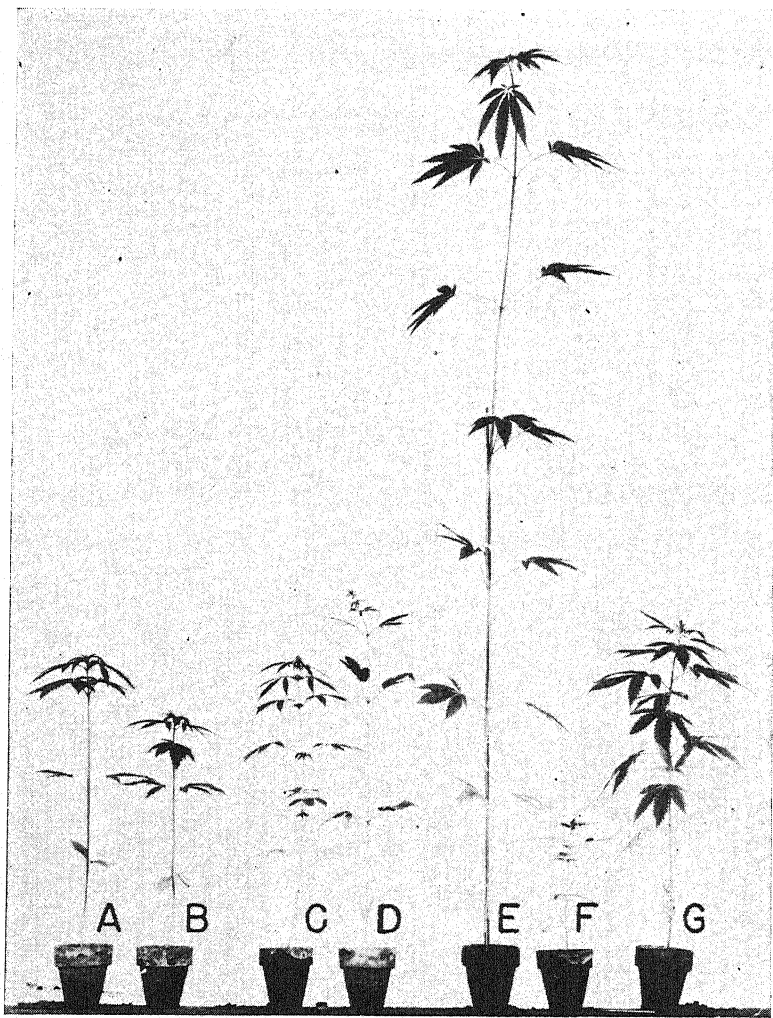


FIG. 7. Top-root ratios of Hemp, var. Femarrington. Numbers are top-root ratios. A, cool, short photoperiod (budding) 6.4; B, cool, long photoperiod (vegetative) 4.9; C, medium, short photoperiod (female blossoms) 7.4; D, medium, short photoperiod (male blossoms) 14.5; E, medium, long photoperiod (vegetative) 8.3; F, warm, short photoperiod (female) 9.5; G, warm, long photoperiod (budding) 9.2. Not pictured, warm, short photoperiod (male) 20.2.

#### EFFECT OF TEMPERATURE UPON TOP-ROOT RATIO

Again from table I, it is seen that many of the plants had greater top-root ratios in a warm temperature. Among these are spinach, stock, blue

grass, cabbage, Cineraria, German millet, Jap buckwheat, Nicandra and Prince of Wales peas. On the other hand some plants as geranium and the phlox, Miss Lingard, have a larger top-root ratio in a cool temperature. The other species under study showed no consistent response to influence of temperature.

#### EFFECT OF FLOWERING UPON TOP-ROOT RATIO

Since neither photoperiod nor temperature were consistently related to top-root ratio throughout the list of plants being grown, the question remained as to whether the formation of blossoms and fruits was associated with a greater top-root ratio at a given temperature, in the case of those kinds which show a consistent temperature influence. The data of table I, as well as the data for hemp, show a consistently greater amount of top in relation to the root in the case of flowering plants. From figures 3-5 as well as successive samplings of several other lots as the season progressed it is clear that the high top-root ratio was not the result of the photoperiod and temperature combination in which the plants were growing but was a condition which arose as the plants developed flowers and fruits. It is thus clear that coming to flower reduces root extension in relation to the weight of top produced.

#### Discussion

The data presented consistently show an effect of the environment surrounding the top upon the top-root ratio. This is also consistent with the

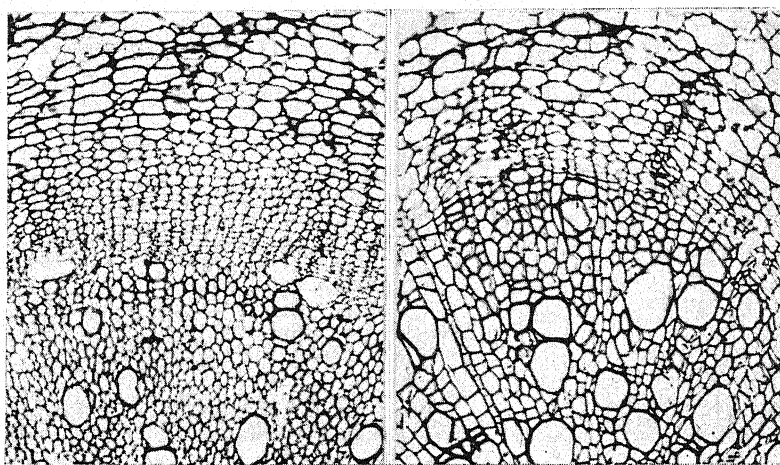


FIG. 8. Cross-sections of roots of tobacco var. Maryland Mammoth. A, nonflowering plant; B, flowering plant. Changes in structure accompanying flower production are comparable to those found in the stem.

well-known influence of shade, partial defoliation, and girdling (of some species) in reducing root extension. The characteristic differences in amount of root formation and top-root ratio of different varieties are marked. Also the influence of blossom induction and fruiting in checking root growth pre-

sents a problem when considering the rôle and efficiency of root systems. The question which is particularly raised is—at what stage of growth should samples be taken.

The effect of flowering on the top-root ratio may well be related to the reduced cambial activity and limited development of phloem tissue which accompanies blossoming (8). Partially completed studies of the relation of flowering to root anatomy have shown that the structure of the roots of annuals is similarly modified by the reproductive state of the top (fig. 8). The question arises naturally as to the possibility of altered transport following a reduction in phloem development being involved in reduced root growth which follows from shading, partial defoliation or girdling.

The fact that an environmental factor as length of photoperiod or temperature does not have a consistent effect upon top-root ratio points to the possibility that the internal conditions of the plant as elaborated or storage material, rather than purely external factors would be the real determinants of root production. This could even be the case, as CURTIS (3) has suggested, where soil conditions are credited with influence upon the roots. The composition and make-up of the top as an influence upon roots is also indicated by the seasonal changes in top-root ratio of plants under uniform soil, temperature and light conditions.

All of these observations combine to raise a question as to the essential value of studies of the top-root ratio when attempting to determine the rôle and function of roots in plant growth and development. It appears that the "efficiency" of a root system must be measured by other techniques than recording top-root ratio; possibly by such studies as those by WENT (12). He has concluded "that less than ten per cent. of the root system . . . is responsible for more than 50 per cent. of their (tomato) growth rate."

### Summary

Twenty-nine species or varieties of plants were grown in long and short photoperiods at minimum (night) temperature of 55°, 65°, and 75° F. in a uniform soil.

Different species have characteristic top-root relations.

The top-root ratio was found to vary with changes in the physiological conditions associated with the seasonal development of the plant.

A given external environment such as length of photoperiod or temperature was found not to have a similar effect upon the top-root ratio of different species. Many but not all of the species grown have more roots in relation to the top in cooler temperatures than in warm. Some have fewer roots in relation to top when the temperature is cool. Likewise, some kinds have smaller top-root ratios in short photoperiods and others in long photoperiods.

The top-root ratio was uniformly larger when the plants came to flower; that is, blossoming plants consistently have fewer roots in relation to the amount of top at a given temperature than non-flowering plants of a species.

It appears that the composition and reserve conditions within the top is obviously a large, if not the controlling, factor in the production of roots and so, the top-root ratio.



A measurement of the top-root ratio would seem to offer less than is sometimes expected towards a solution of the problem of the rôle or efficiency of roots in plant growth.

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## SELECTIVITY OF HERBICIDES

A. S. CRAFTS

With the introduction of new organic chemicals, the use of selective herbicides is finding broader application. Stimulated by war conditions, their use offers a promising method for increasing efficiency and reducing drudgery of agricultural production.

Early work on selective herbicides is reviewed by ÅSLANDER (1), and by ROBBINS, CRAFTS, and RAYNOR (17). This paper considers selectivity from a broader viewpoint, discussing the selective killing of annual weeds in crops of the family Umbelliferae by oils, the control of annuals and shallow-rooted perennials among deep-rooted perennial crops, and the selective use of growth regulators as sprays and soil amendments.

Plants differ in many ways; their surfaces vary in form and chemical composition; some are easily wet by aqueous sprays; others are difficult to wet. Surface coatings vary in permeability, and protoplasm responds specifically to different chemicals. The gross structure of plants varies in such a way that the meristematic tissues of some are exposed and vulnerable to sprays, others have their growing points protected. Because of these differences certain weeds may be destroyed in growing crops with little or no injury to the latter.

### The nature of selectivity

The surface of cereal leaves is usually minutely ridged, that of broad-leaved weeds such as Brassica, Raphanus, and Amsinckia species is smooth. The cuticle of cereal leaves is silicious, waxy, or coated with minute particles of waxy bloom; that of the above weeds is more susceptible to wetting with aqueous sprays. The leaves of cereal crops tend to be upright; those of broad-leaved weeds are flat and horizontal. The growing points of cereals are located at the base of the plant and are protected by older leaves; the same applies to onion. Growing points of broad-leaved weeds are terminal and exposed.

Roots of many annual and shallow-rooted perennial weeds are located in the top soil layer; those of deep-rooted plants may extend many feet into the subsoil. Examples are foxtail (*Hordeum* spp.), shepherd's purse (*Capsella bursa-pastoris*), plantains (*Plantago* spp.), and chicory (*Cichorium intybus*) as weeds in alfalfa. Finally, differences in the susceptibility of weeds and crops depending upon the reactivity of the protoplasm may be illustrated by annual blue grass (*Poa annua*), mustards, and shepherd's purse; such weeds are commonly killed by stove oil,<sup>1</sup> whereas carrots and celery are unharmed.

<sup>1</sup> Stove oil is a light fuel oil used for domestic heating in the Western United States. It is an unrefined fraction of western crudes conforming to the following specifications: Gravity, API (Amer. Petrol. Inst.) 37.6° to 38.7°.

Viscosity (Saybolt Universal) at 100° F., 31 to 33 secs.

Flash point (Pensky-Martens closed cup) 134°-140° F.



All these plants are thoroughly wet by the spray, which penetrates the carrot and celery plants, as evidenced by their oily flavor. Evidently the selectivity shown between grasses and broad-leaved plants by the growth-regulating chemicals is of the same nature; it is displayed both by the salts of dichlorophenoxyacetic acid in aqueous solution and by the esters in nontoxic oil. Selectivity is shown by plants both after treatment of the roots through the soil (18) and after spraying of the tops (9, 14).

The action of selective herbicides is relative, depending on the concentration or dosage applied. No case of absolute selectivity is known; the toxicant will always kill both weed and crop species if brought into intimate contact with the plants in sufficient concentration. Herbicides known for their general contact toxicity (ability to kill all plant species) are selective if applied to weeds and crop plants in a series of concentrations.

### Experimental work

Data presented are samples from much experimentation over several years. They have been selected to illustrate the general principles just presented. Thus the problem of relative toxicity as compared with absolute or universal toxicity was investigated. Early work (4) proved the efficacy of sodium chloride, iron sulfate, copper sulfate, and even sodium arsenite as selective herbicides. On the other hand, sodium arsenite was used as a general contact "weed killer" (21) and soil sterilant (12, 19). Evidently arsenic could serve as either a selective or a general contact herbicide. Studies on selective herbicides in cereal-crop experiments at Davis, California, in the spring of 1932 included plots sprayed with sodium arsenite solutions (table I). The plants averaged about 6 inches in height at the time of spraying.

The 4 per cent. solution killed all the barley and weeds. The 2 per cent. solution killed all the weeds and injured the barley, but the latter recovered rather quickly. The 1 per cent. solution injured the barley slightly and failed to eliminate fiddleneck completely. The lower concentrations injured the weeds but did not control them sufficiently to prevent reseeding. Plots 2 and 3 had materially increased yields; plot 4 was somewhat better than the checks.

Evidently sodium arsenite, ordinarily regarded as a general contact weed killer, will act selectively if applied at the proper concentration.

Practically all inorganic salts are somewhat toxic to plants if sufficiently concentrated solutions are applied to the foliage. Many are low in toxicity and will not kill all species, even if applied in saturated solution; most of

#### Distillation temperatures:

Initial boiling point .....	335° to 380° F.
10 per cent. point .....	380° to 390° F.
90 per cent. point .....	480° to 515° F.
End point .....	535° to 570° F.
Water and sediment .....	0.0 to trace
Sulfur content .....	0.13% to 0.68%

these would be of no practical value as herbicides. The following list enumerates salts that have been tried and found useful: iron sulfate, copper sulfate, copper chloride, copper nitrate, sodium chloride, sodium chlorate, sodium arsenite, barium chlorate, sodium chromate, sodium dichromate, sodium nitrate, sodium bisulfate, nickel sulfate, ammonium sulfate, zinc sulfate, potassium chloride, magnesium chloride.

Besides these, many other salts have been tested (5). Arsenic acid and sulfuric acid have also given practical results; in fact, for several years before the dinitro compounds were introduced, sulfuric acid led as a selective herbicide, particularly in cereal crops.

TABLE I

TOXICITY OF SODIUM ARSENITE SPRAYS TO MUSTARD, FIDDLENECK,\* AND BARLEY.  
INJURY IS EXPRESSED AS PERCENTAGE OF DAMAGE TO FOLIAGE COMPARED  
WITH UNTREATED CHECKS. ALL PLOTS 1 SQUARE ROD IN AREA.  
TREATMENT FEBRUARY 19, 1932

PLOT NO.	ARSENIC CONCEN- TRATION†	INJURY BY MARCH 1, 1932			INJURY BY APRIL 1, 1932		
		BARLEY	MUSTARD	FIDDLE- NECK	BARLEY	MUSTARD	FIDDLE- NECK
	%	%	%	%	%	%	%
1	4.00	100	100	100	100	100	100
2	2.00	10	100	100	0	100	100
3	1.00	5	100	90	0	100	90
4	0.50	0	95	85	0	95	75
5	0.25	0	90	75	0	90	50
6	0.125	0	75	10	0	50	0

\* The common name fiddleneck is used here to designate *Amsinckia douglasiana*, a common grainfield weed of central California.

† Percentage of  $\text{As}_2\text{O}_3$  by weight.

Selectivity of these salts and acids in aqueous solution is largely a result of differential wetting; differences in the surface coating of the plants and in the form and orientation of leaves, rather than chemical tolerances, account for the differences in injury. If wetting agents are added to such solutions, selectivity is reduced; the crop plants may be severely injured.

In addition to the protection afforded plants because of their ability to resist wetting, other mechanisms may be involved. For instance, if wetting agents are added to sulfuric acid sprays, grass leaves will be severely damaged; stems, on the other hand, may show little injury; and new leaves, developing from growing points buried deeply in the centers of the stems near the crown, may be unharmed. This type of selectivity, dependent upon structure, became evident when wetting agents were added to sulfuric acid in an effort to develop a general contact herbicide. Besides grasses, *Erodium* species in the rosette showed resistance; and in this case, again, selectivity was based upon structure, not upon wettability or any other chemical characteristic. As with sodium arsenite, sulfuric acid will kill all plants if applied in sufficient concentration; at intermediate concentrations (about 10 per

cent. by weight) it readily kills broad-leaved plants but not grasses; and at low concentrations (about 3 per cent.) it will kill dodder, small seedlings of mustards, chickweed, and the like. Toxicity again ranges from total to zero, depending upon concentration, plant species, and environmental factors that determine the susceptibility of plants to acid injury.

#### THE DINITRO COMPOUNDS

The introduction of Sinox (the sodium salt of dinitro ortho cresol) in 1938 started a new phase in the use of selective herbicides. Experiments in California (20) proved this material to be highly selective, noncorrosive, and far less toxic to animals than sodium arsenite (16). Soon after its commercial introduction, Sinox was used extensively on the Pacific Coast. In Oregon (10) it was tried in combination with other chemicals, notably ammonium sulfate, calcium cyanamid, sodium bisulfate, sulfuric acid, sulphamic acid, and ammonium sulfamate. These supplementary chemicals were first added to test the possibility of applying a fertilizer along with the herbicide. All those listed above increased the effectiveness of Sinox, ammonium sulfate producing the most outstanding results. It soon became evident that an amount of this chemical insufficient to give a fertilizing effect greatly increased the activity of the Sinox, and that crop increases were incommensurate with the total nitrogen applied. Furthermore, comparable results were obtained by the use of sodium bisulfate. Since toxicity of sodium dinitro ortho cresylate was increased when these acid salts were added, such addition soon became known as "activation."

TABLE II

CROPS IN WHICH SELECTIVE WEED CONTROL MAY BE PRACTICED, AND WEEDS THAT MAY BE KILLED BY SELECTIVE HERBICIDES IN AQUEOUS SOLUTION

CROPS	
Wheat	Corn
Barley	Onions
Oats	Garlic
Rye	Ryegrass
Flax	Fescue
Peas	
WEEDS	
Mustards, <i>Brassica nigra</i> and <i>B. arvensis</i>	
Wild turnip, <i>Brassica campestris</i>	
Hedge mustard, <i>Sisymbrium</i> spp.	
Fiddleneck, <i>Amsinckia</i> spp.	
Fan weed, <i>Thlaspi arvense</i>	
Pennycress, <i>Thlaspi perfoliatum</i>	
Russian thistle, <i>Salsola kali</i>	
Prickly lettuce, <i>Lactuca scariola</i>	
Corncockle, <i>Agrostemma githago</i>	
Shepherd's purse, <i>Capsella bursa pastoris</i>	
Small nettle, <i>Urtica urens</i>	
Nightshade, <i>Solanum nigrum</i>	
Wild buckwheat, <i>Polygonum convolvulus</i>	
Lamb's-quarter, <i>Chenopodium album</i>	
Hungerweed, <i>Ranunculus arvensis</i>	

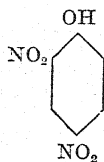
In California, work on the activation of Sinox (7) has indicated the nature of the chemical reaction involved. Besides demonstrating that Sinox could be activated, HARRIS and HYSLOP (10) showed conclusively that ammonium sulfate or calcium cyanamid could be applied simultaneously with the selective herbicide and that the enhanced toxicity resulted in excellent control of weeds, including some species which Sinox would ordinarily not affect. They also showed that sulfamic acid and ammonium sulfamate would activate Sinox. More recently aluminum sulfate has been added to the list of activators.

Not only have the field trials demonstrated the efficiency of Sinox as a selective herbicide; they have increased the number of crops in which this kind of control may be practiced. Table II lists the known crops in which weeds may be treated selectively and the principal weeds that may be killed by selective herbicides. As this list shows, many new crops have been added since Bolley's early experiments with selective herbicides. The question naturally arises: how far can this list be extended? There are two chief ways of improving selective herbicides: (1) by increasing the toxicity so that dosage can be reduced, and (2) by widening the selectivity or extending it to entirely new groups of plants. Both these improvements have been accomplished in recent years.

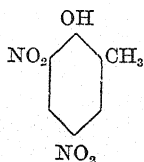
#### INCREASED TOXICITY

The first step in increasing the toxicity of the dinitro compounds was the use of acid salts as activators in the way mentioned above. The next step was to test the ammonium salt of dinitro ortho cresol. It proved as toxic as the activated sodium salt. Since the ammonium salt is not as highly inflammable as the dry sodium salt, it is a water-soluble dry powder that may be used under field conditions. Its use reduces the weight of material required per acre from 10 pounds (the approximate weight of 1 gallon of Sinox) to 3 pounds. This effects a real saving in transportation and provides a more convenient form for handling the chemical.

Dinitro phenol has the formula



The formula of dinitro ortho cresol is



Since the latter is much more toxic than the phenol, it seems reasonable that lengthening the aliphatic chain might further increase toxicity. This has

proved to be true. Testing of the 2,4 dinitro compounds of phenol, o-methyl, o-ethyl, o-isopropyl, o-secondary butyl, and o-secondary amyl phenol, respectively, showed that toxicity increases through the first five and drops slightly in the sixth. Table III from CRAFTS (6) illustrates this relation. The ammonium salts of these compounds are water-soluble, and ammonium dinitro ortho secondary butyl phenylate is three to four times as toxic as the corresponding cresylate. It is less irritating than the cresylate, readily soluble in water, and its density is such that  $\frac{3}{4}$  pound finely powdered has a volume of about 1 pint. This, dissolved in 100 gallons of water, will control mustards and similar grain-field weeds in an average acre of grain. Further increases in the toxicity of selective herbicides will be of little benefit unless accompanied by a decrease in cost because the volume of material mentioned above is about as low as is convenient to handle.

TABLE III

SOLUBILITY IN OIL AND RELATIVE TOXICITY\* OF DINITRO COMPOUNDS†

CHEMICAL	SOLUBILITY‡ IN KEROSENE AT 20° C.	TOXICITY*	$\frac{\text{CHAIN WEIGHT}}{\text{TOTAL MW}} \times 100$
	% (approx.)		
Dinitro phenol .....	0.14	38	0.0
Dinitro-o-cresol .....	0.58	64	7.5
Dinitro-o-ethyl phenol .....	2.39	75	13.6
Dinitro-o-isopropyl phenol .....	3.43	90	18.9
Dinitro-o-secondary butyl phenol .....	Miscible	100	23.6
Dinitro-o-secondary amyl phenol .....	Miscible	90	27.8

\* Toxicity in arbitrary units has been calculated to a basis of 100 per cent. for dinitro-o-secondary butyl phenol, the most toxic of the six compounds. This toxicity would require concentrations of approximately 0.5 per cent. on grasses and 0.125 per cent. on broad-leaved weeds if applied in nontoxic oil or oil emulsion under the conditions of these experiments.

† Table taken from CRAFTS (6).

‡ Solubility is higher in more polar solvents. The aromatic and olefin contents of petroleum fractions largely determine their solvent power for the nitrophenols.

#### BROADENING SELECTIVITY; THE SELECTIVE OILS<sup>2</sup>

Discovery of the selective action of certain petroleum fractions has added a whole new group of crops that may be weeded chemically; those belonging to the family Umbelliferae. Principal among these are carrots and celery; others include parsnip, parsley, dill, and caraway. Guayule will tolerate stove oil and low dosages of diesel oil.<sup>3</sup>

<sup>2</sup> Work on the herbicidal properties of oil in California has been cooperative between the Botany and Chemistry Divisions of the College of Agriculture. H. G. REIBER and H. W. ALLINGER of the Chemistry Division have aided in the experimental work reported here.

<sup>3</sup> Diesel oil is a medium grade fuel oil, used for diesel motors. It is not highly refined and it is toxic to most plants. The following specifications are used in purchasing diesel oil for spraying fire strips along California highways:

Gravity (A.P.I.) at 60° not less than 27° F.

Viscosity (Saybolt Universal) at 100° F. not over 50 secs.



Selectivity of petroleum oils is evident among weeds as well as crop plants. Fennel (*Foeniculum vulgare*), snake root (*Sanicula* spp.) and poison hemlock (*Conium maculatum*) are three Umbelliferae that escape injury from oil. Pineapple weed (*Matricaria suaveolens*), groundsel (*Senecio vulgaris*), purslane (*Portulaca oleracea*), and yellow star thistle (*Centaurea solstitialis*) are somewhat tolerant, especially in the rosette stage. Grasses and many broad-leaved annuals are readily killed by spraying with oils.

According to studies on the toxicity of oils, the aliphatic hydrocarbons are nontoxic; the aromatic hydrocarbons and olefinic compounds are largely responsible for oil injury (8). Of the aromatic and olefinic compounds that occur naturally in petroleum, those boiling below about 200° F. cause a rapid killing of plant tissue, termed acute toxicity; those boiling above 200° F. act more slowly but cause a more profound injury—the so-called chronic toxicity (8). Selective toxicity of the type caused by stove oil is largely acute; and experiments have shown that oil fractions even lighter than stove oil are preferable as carrot sprays because they are more toxic and leave less residual odor and flavor in the vegetable.

There is no sharp point of distinction between compounds causing acute and chronic injury. The temperature of 200° F. represents the low point of a range. Compounds boiling below 200° F. show acute toxicity; those boiling above about 400° F. show chronic toxicity. In the range between 200° and 400° F., compounds will exhibit some of each type provided the dosage is adjusted so that acute toxicity will not kill the plants.

Selectivity and toxicity of oil fractions vary with the boiling range. Carrots tolerate the light oils boiling below 200° F. Under similar growing conditions, flax and onions are injured by such light oils, but the latter crops will tolerate low concentrations of aromatic and olefinic toxicants in the boiling range of kerosene, namely 200° to 400° F. For this reason kerosene will not injure flax and onions; but even its low content of chronic toxicant is sufficient to kill seedling grasses such as wild oats and barley. One can therefore obtain selective killing of these grasses in flax and onions in the greenhouse at will. In some cultures the same grasses were killed without injury to wild mustard. This is the reverse of the selectivity shown by dinitro compounds. In fact, the oils seem to have a specific selectivity against grass species, in contrast to broad-leaved plants.

Certain plants tolerate the chronic toxicants of oils as heavy as diesel oil. Notable among these are guayule and fennel. Light dosages of diesel oil are regularly used for controlling weeds in guayule crops. Where this oil has been used annually to kill weeds on fire strips along highways in California, fennel has been uninjured and has grown, unlimited by competition, occupying many sprayed areas completely.

Selective toxicity of oils differs from that of aqueous solutions because

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Flash point (PMCC) not less than 150° F.

Distillation, 90% point not over 680° F.

Water and sediment not over a trace.



wetting is not involved. Since the tops of the plants are thoroughly wet by the oil sprays, the absence of injury to the tolerant crops and weeds must be characteristic of their protoplasm.

Selectivity of oils, again, is relative; if the toxicant is used in high enough concentration, all plants are killed. This point was proved experimentally in two ways.

TABLE IV

TOXICITY OF XYLENE, ISOPARAFFIN, AND THEIR COMBINATIONS TO CARROTS, MUSTARD, AND FOXTAIL (*Hordeum murinum*). DATE OF APPLICATION  
MARCH 14, 1945, 9:00-10:00 A.M.

OIL MIXTURE		INJURY—PERCENTAGE OF UNTREATED CHECKS					
XYLENE	ISO-PARAFFIN	DATE OF OBSERVATION					
		MARCH 14, 1945			MARCH 15, 1945		
		CARROTS	MUSTARD	FOXTAIL	CARROTS	MUSTARD	FOXTAIL
%	%	%	%	%	%	%	%
100	0	95	100	100	100	100	100
80	20	90	95	100	95	100	100
60	40	80	95	95	85	95	95
50	50	20	95	90	25	95	95
40	60	5	95	75	5	95	80
0	100	0	0	0	0	0	0
		MARCH 16, 1945			MARCH 17, 1945		
100	0	100	100	100	100	100	100
80	20	95	100	100	95	100	100
60	40	85	95	100	85	100	98
50	50	35	95	98	40	95	98
40	60	5	95	90	5	95	95
0	100	0	0	0	0	0	0
		MARCH 19, 1945			MARCH 21, 1945		
100	0	100	100	100	100	100	100
80	20	90	100	100	90	100	100
60	40	75	100	95	75	100	95
50	50	40	95	100	40	100	100
40	60	5	98	95	5	98	98
0	100	0	0	0	0	0	0
		MARCH 26, 1945			MARCH 31, 1945		
100	0	100	100	100	100	100	100
80	20	80	100	99	60	100	99
60	40	70	100	98	50	100	98
50	50	30	98	100	15	98	100
40	60	0	98	98	0	95	99
0	100	0	0	10	0	0	10

First, the aromatic compound, xylene, was used straight, and also in a series of dilutions with a relatively nontoxic aliphatic hydrocarbon. Either pure xylene or xylene in concentrations above 60 per cent. was fatal to all plants. At concentrations between 60 and 40 per cent., it killed weeds without injuring carrots. Table IV presents the results of one such experiment.

Second, aromatic and olefinic compounds extracted from petroleum fractions were used pure and in dilution series. The results were similar to those reported with xylene (tables V and VI). These petroleum extracts, however, are higher in toxicity than xylene. For this reason, straight gasoline containing around 20 per cent. of aromatic and olefinic compounds is highly selective whereas xylene, to produce the same results, must be used at a higher concentration.

TABLE V

TOXICITY OF OLEFINIC FRACTION IN DILUTION WITH NORMAL CETANE TO CARROTS, FLAX, AND FOXTAIL. DATE OF APPLICATION APRIL 25, 1945

OIL MIXTURE		INJURY—PERCENTAGE OF UNTREATED CHECKS					
HEAVY OLEFINIC FRACTION	CETANE	APRIL 26, 1945			APRIL 28, 1945		
		CARROTS	FLAX	FOXTAIL	CARROTS	FLAX	FOXTAIL
%	%	%	%	%	%	%	%
50	50	5	20	50	10	50	85
45	55	5	10	30	20	40	70
40	60	0	5	20	5	20	60
35	65	0	0	10	0	10	30
30	70	0	0	5	0	2	20
25	75	0	0	0	0	1	10
		MAY 1, 1945			MAY 3, 1945		
50	50	20	90	100	10	95	100
45	55	20	75	80	15	90	90
40	60	10	65	75	10	75	80
35	65	0	35	30	0	40	40
30	70	0	10	25	0	10	20
25	75	0	5	15	0	5	10
		MAY 5, 1945			MAY 7, 1945		
50	50	10	95	100	15	95	100
45	55	15	90	95	15	90	95
40	60	10	80	85	15	80	90
35	65	0	50	75	5	60	80
30	70	0	15	20	0	20	20
25	75	0	5	10	0	5	10
		MAY 11, 1945			MAY 14, 1945		
50	50	15	95	100	10	90	100
45	55	10	90	100	5	80	100
40	60	10	80	95	5	70	100
35	65	0	60	90	0	50	100
30	70	0	15	40	0	10	75
25	75	0	0	20	0	0	40

The selective nature of stove oil is purely fortuitous. It depends on the fact that this fraction is within a boiling range which largely excludes compounds toxic to carrots. Stove oil for carrot spraying should have an API gravity rating of  $38.0 \pm 0$  F. A sample of such oil rating  $34.7^\circ$  severely injured carrots in the field. This observation proves that stove oil is just at the limit of gravity for carrot spraying. A lighter oil is superior; not

only does it have higher selectivity, but it is more volatile and hence leaves less objectionable odor and flavor. Experiments with third-structure gasoline as a carrot spray have proved these statements to be correct. Third-structure gasoline is the heaviest and consequently the least expensive grade sold commercially as an automobile fuel.

In the Eastern United States no fraction comparable with stove oil is manufactured. In attempting to duplicate such a fraction, LACHMAN (13)

TABLE VI

TOXICITY OF HEAVY AROMATIC FRACTION IN DILUTION WITH NORMAL CETANE TO CARROTS, FLAX, AND FOXTAIL. DATE OF APPLICATION APRIL 25, 1945

OIL MIXTURE		INJURY—PERCENTAGE OF UNTREATED CHECKS					
HEAVY AROMATIC FRACTION	CETANE	APRIL 26, 1945			APRIL 28, 1945		
		CARROTS	FLAX	FOXTAIL	CARROTS	FLAX	FOXTAIL
%	%	%	%	%	%	%	%
40	60	5	50	50	10	85	90
35	65	0	5	60	5	25	90
30	70	0	0	30	0	10	60
25	75	0	0	15	0	10	40
20	80	0	0	10	0	5	25
0	100	0	0	0	0	0	10
		MAY 1, 1945			MAY 3, 1945		
40	60	15	95	100	15	95	100
35	65	5	80	100	5	80	100
30	70	0	70	90	0	70	95
25	75	0	50	75	0	60	80
20	80	0	20	50	0	20	60
0	100	0	0	0	0	0	0
		MAY 5, 1945			MAY 7, 1945		
40	60	15	95	100	10	95	100
35	65	5	80	100	5	80	100
30	70	0	70	95	5	70	100
25	75	0	60	85	0	60	90
20	80	0	20	75	0	25	80
0	100	0	0	0	0	0	0
		MAY 11, 1945			MAY 14, 1945		
40	60	10	95	100	5	90	100
35	65	5	90	100	0	90	100
30	70	5	75	100	0	75	100
25	75	0	60	90	0	60	75
20	80	0	25	80	0	25	50
0	100	0	0	0	0	0	0

recommends oils of the "straight-run petroleum naphtha type" having "an aromatic content of approximately 15 per cent." Some such oils are "Sovasol No. 5," "Stoddard Solvent," "Mineral spirits," and "Sun Spirits." These are fractions used in industry as paint thinners and cleaning solvents. Though considerably more expensive than stove oil, they are still economical where they can be used in place of hand labor.

## SELECTIVITY THROUGH THE SOIL

The selective herbicides discussed above have all been contact sprays that produce selective action on the tops of plants. Many compounds having the properties of soil sterilants act selectively upon plants through the roots; in fact selectivity among plants is commonly manifested, and only when heavy dosages are used is sterilization against all weeds obtained.

With sterilants of the volatile type, loss of vapor from the top soil may result in selectivity. For instance, with carbon disulfide one may destroy the wild morning-glory in an area without killing Bermuda grass, which is relatively shallow-rooted. Such a treatment might be used to kill deep-rooted perennials in a Bermuda grass lawn.

In the use of the temporary sterilant sodium chlorate, selectivity may follow from localization of the chemical in the soil, or it may result from a chemical tolerance shown by certain plants of saline regions. Of the first type is the killing of Johnson grass by a winter application of chlorate to the soil, and the survival of wild morning-glory in the same area; the chemical becomes diluted before it can percolate to the deep roots of the latter pest. Another instance, noted many times, is the survival of poison oak (*Rhus diversiloba*) on firebreaks where chlorate has been used alone or in combination with arsenic to control bear-mat (*Chamaebatia foliolosa*) and other shallow-rooted perennials.

As an example of chlorate tolerance, salt grass (*Distichlis spicata*) cannot be eradicated easily by this chemical although Bermuda grass (*Cynodon dactylon*), Russian knapweed (*Centaurea repens*), and other perennials growing on the same area will be killed. Alkali-tolerant weeds such as salt-bush (*Atriplex semibaccata*), bractscale (*A. bracteosa*), and spikeweed (*Centromadia pungens*) are usually the first weeds to reappear where chlorate has been used to kill Johnson grass (*Holcus halepensis*), wild morning-glory (*Convolvulus arvensis*), or other perennials.

Despite the extreme toxicity of arsenic, certain weed species, notably bractscale, yellow star thistle, salt grass, and similar salt-tolerant pests, may grow where the soil has been made completely sterile to other plants.

Borax has an advantage over chlorate and arsenic, in that grasses are stimulated by intermediate concentrations (6 lbs. per square rod) of it in the soil (17). The same concentrations are toxic to St. Johnswort (*Hypericum perforatum*) and bear-mat. After borax application for control of these pests, grasses usually invade the treated areas to the total exclusion of the weeds, and growth is often more vigorous than on normally weed-free grass areas. Because selective chemicals may thus shift vegetation types, conceivably they might find extensive use in range-improvement work.

## GROWTH-REGULATING SUBSTANCES AS SELECTIVE WEED KILLERS

A wholly new field in chemical weed control has been opened by the discovery that plant-growth-regulating substances may be used as selective weed killers—a discovery made in Britain in 1942 by SLADE, TEMPLEMAN,

and SEXTON (18) and in this country in 1944 by BEAL (2), MITCHELL and HAMNER (15), and HAMNER and TUKEY (9). As these workers have shown, cereal and grass crops exhibit considerable tolerance for this type of chemical, whereas most broad-leaved plants, including many weed species, are highly susceptible. Consequently, the growth regulators can be used selectively to control broad-leaved weeds in cereal crops, pastures, lawns, and elsewhere.

Furthermore, selective action is shown by the roots from application of the chemical to the soil, as well as by the tops after a spray application on the foliage. The outstanding feature of this growth-regulating type of chemical is its extreme toxicity; satisfactory control of mustard species has been secured with  $\frac{1}{2}$  to 1 pound per acre, and BLACKMAN (3) reports almost complete control of *Brassica arvensis* in the seedling stage with 4 ounces per acre applied as a spray. Cereal crops produce increased yields through lack of competition with the weeds.

After being sprayed on, the growth regulators are absorbed through the leaves. They are translocated apparently in the same way as naturally occurring plant hormones; that is, in a polar fashion from foliage to roots. Being toxic, they will kill the perennial root systems of many weeds such as wild morning-glory, Canada thistle (*Cirsium arvense*), dandelion (*Taraxacum vulgare*), and plantain. They can therefore be used to control practically all broad-leaved weeds in lawns, pastures, and cereal and grass crops. They are valuable against poison ivy (*Rhus toxicodendron*), poison oak, poison sumac (*Rhus vernix*), Japanese honeysuckle (*Lonicera japonica*), and similar pests in pasture or woodland areas. Recent tests indicate their use in eliminating cattails (*Typha* spp.), tules (*Scirpus* spp.), bur-reed (*Sparganium eurycarpum*), willows, and other weeds of wet lands and drainage channels (11). Chief among such chemicals are the phenoxyacetic acid derivatives. In Britain the 4 chloro-2 methyl compound has found most favor; in this country the 2,4 dichloro compound. Both the phenoxy and naphthoxy compounds are effective, and the sodium and ammonium salts of the acetic acid derivatives have been used.

Although the growth regulators show their greatest selectivity between grasses and broad-leaved species, other plants display varying susceptibilities to their toxic action. Weeds as well as crop plants differ, and some differences occur among the grasses. Table VII lists a number of weeds whose reaction to growth regulators has been noted. In using this table one should remember that small seedlings are easier to kill than mature plants; that perennials in active growth are more susceptible than when old and woody; and that the form of the chemical, the dosage, and the method of application may affect the result.

Woody plants sprayed in July and August by HAMNER and TUKEY (9) developed growth curvatures; the leaves dropped off, and the stem tips were killed. As those workers note, treatment earlier in the season may give more drastic results.

In instances where the same species were involved, results of tests in California substantially agree with those cited above. The California observations have been made on plot tests where 2,4-D (2,4 dichlorophenoxyacetic

TABLE VII

WEEDS THAT HAVE PROVED SUSCEPTIBLE OR RESISTANT TO GROWTH REGULATORS\*

INTERMEDIATE SPECIES	REFERENCE	INTERMEDIATE SPECIES	REFERENCE
<i>Alsine (Stellaria) media</i> .....	22	<i>Leptilon canadense</i> .....	22
<i>Anthemis cotula</i> .....	22	<i>Oxalis corniculata</i> .....	23
<i>Cardaria</i> spp. ....	23	<i>Plantago major</i> .....	22
<i>Chenopodium album</i> .....	22	“ <i>rugelii</i> .....	22
“ <i>glaucum</i> .....	22	<i>Polygonum aviculare</i> .....	22, 23
<i>Euphorbia maculata</i> .....	23	<i>Rumex Acetosella</i> .....	23
<i>Hypericum perforatum</i> .....	23	<i>Solidago</i> spp. ....	23
<i>Lactuca scariola</i> .....	22	<i>Tragopogon pratensis</i> .....	22
“ spp. ....	22	<i>Typha latifolia</i> .....	23
RESISTANT SPECIES	REFERENCE	RESISTANT SPECIES	REFERENCE
<i>Achillea millefolium</i> .....	14, 22, 23	<i>Matricaria inodora</i> .....	3
<i>Agropyron repens</i> .....	9	<i>Muhlenbergia schreberi</i> .....	22
<i>Agrostema githago</i> .....	22	<i>Oxalis corniculata</i> var.	
<i>Alchemilla arvensis</i> .....	3	“ <i>atropurpurea</i> .....	11, 22, 23
<i>Anthemis cotula</i> .....	18, 23	<i>Persicaria persicaria</i> .....	22
<i>Asclepias speciosa</i> .....	23	“ <i>pennsylvanicum</i> .....	22
<i>Atriplex patula</i> .....	3	<i>Physalis</i> spp. ....	23
<i>Avena fatua</i> .....	9, 11	<i>Plantago major</i> .....	14
“ <i>sativa</i> .....	18	<i>Poa annua</i> .....	11
<i>Bromus mollis</i> .....	11	“ <i>pratensis</i> .....	9
“ <i>rigidus</i> .....	11	<i>Polygonum aviculare</i> .....	3
<i>Cenchrus pauciflorus</i> .....	22	“ <i>convolvulus</i> .....	22
<i>Centaurea repens</i> .....	11, 23	“ <i>tartaricum</i> .....	22
<i>Cephalanthus occidentalis</i> .....	23	<i>Pteris aquilina</i> .....	3, 23
<i>Chaetocloa</i> spp. ....	22	<i>Rhus diversiloba</i> .....	23
<i>Chrysanthemum leucanthemum</i> var. <i>pinnatifidum</i> .....	14	<i>Rubus procerus</i> .....	11
<i>Cynodon dactylon</i> .....	11	“ spp. ....	14, 23
<i>Digitaria ischaemum</i> .....	9	<i>Rumex acetosella</i> .....	14, 22
<i>Digitaria (Syntherisma)</i> <i>sanguinale</i> .....	9, 22	“ <i>obtusifolius</i> .....	14
<i>Echinocloa crus-galli</i> .....	9, 22	“ spp. ....	3, 22
<i>Elusine indica</i> .....	9, 22	<i>Salsola pestifer</i> .....	22
<i>Equisetum</i> spp. ....	22	<i>Saponaria vaccaria</i> .....	22
<i>Euphorbia esula</i> .....	22	<i>Senecio jacobaea</i> .....	23
<i>Fumaria officinalis</i> .....	3	<i>Setaria lutescens</i> .....	9
<i>Helxine soleiroli</i> .....	11	“ <i>viridis</i> .....	9
<i>Holcus halepensis</i> .....	11	<i>Sida hederacea</i> .....	11, 23
<i>Hordeum jubatum</i> .....	22	<i>Silene noctiflora</i> .....	22
<i>Linaria vulgaris</i> .....	23	<i>Solanum nigrum</i> .....	22
<i>Lolium multiflorum</i> .....	11	“ <i>rostratum</i> .....	22
<i>Lychnis alba</i> .....	22	<i>Urtica dioica</i> .....	3
<i>Matricaria chamomilla</i> .....	3	<i>Verbascum thapsus</i> .....	22
		<i>Viola</i> spp. ....	22
SUSCEPTIBLE SPECIES	REFERENCE	SUSCEPTIBLE SPECIES	REFERENCE
<i>Amaranthus blitoides</i> .....	22, 23	<i>Artemisia vulgaris</i> var.	
“ <i>caudatus</i> .....	11	“ <i>heterophylla</i> .....	22
“ <i>retroflexus</i> .....	9, 14, 22, 23	<i>Asclepias syriaca</i> .....	9
<i>Ambrosia artemisiifolia</i> .....	9, 22	<i>Barbarea barbarea</i> .....	22
“ <i>psilostachya</i> .....	11, 23	<i>Brassica arvensis</i> .....	3, 22
<i>Apocynum cannabinum</i> .....	23	“ <i>juncea</i> .....	22
<i>Arctium minus</i> .....	22, 23	“ <i>napus</i> .....	22



TABLE VII (Continued)

WEEDS THAT HAVE PROVED SUSCEPTIBLE OR RESISTANT TO GROWTH REGULATORS\*

SUSCEPTIBLE SPECIES	REFERENCE	SUSCEPTIBLE SPECIES	REFERENCE
<i>Brassica nigra</i> .....	11	<i>Melilotus alba</i> .....	9
" <i>sinapis visiani</i> .....	19	" spp. ....	22, 23
" spp. ....	23	<i>Neslia paniculata</i> .....	22
<i>Camelina</i> spp. ....	22	<i>Oxalis</i> spp. ....	14
<i>Capsella</i> ( <i>Bursa</i> ) <i>bursa-</i>		<i>Papaver Rhoeas</i> .....	3, 18
<i>pastoris</i> .....	14, 22, 23	<i>Plantago lanceolata</i> .....	9, 14, 22
<i>Cardaria draba</i> var. <i>repens</i> ..	11	" <i>major</i> .....	9, 18
<i>Centaurea cyanus</i> .....	3	" spp. ....	23
" <i>solstitialis</i> .....	11, 23	<i>Polygonum aviculare</i> .....	14
<i>Cerastium vulgatum</i> .....	22	" <i>coccineum</i> .....	11
<i>Chenopodium album</i> .....	9, 18, 23	" <i>convolvulus</i> .....	3
<i>Chrysanthemum segetum</i> .....	18	" <i>pennsylvanicum</i> .....	9
<i>Cichorium intybus</i> .....	11, 23	<i>Portulaca oleracea</i> .....	9, 23
<i>Cicuta</i> spp. ....	23	<i>Potentilla</i> spp. ....	22
<i>Cirsium lanceolatum</i> .....	23	<i>Prunella vulgaris</i> .....	23
<i>Conium maculatum</i> .....	11, 23	<i>Ranunculus arvensis</i> .....	3, 18
<i>Conringia orientalis</i> .....	22	<i>Raphanus raphanistrum</i> .....	3, 18
<i>Convolvulus arvensis</i> .....	9, 11, 23	" <i>sativus</i> .....	23
" <i>sepium</i> .....	14	<i>Roripa austriaca</i> .....	23
<i>Datura stramonium</i> .....	14	<i>Rumex acetosella</i> .....	11
<i>Daucus Carota</i> .....	23	" <i>crispus</i> .....	23
<i>Eichornia crassipes</i> .....	11	<i>Salix</i> spp. ....	23
<i>Equisetum</i> spp. ....	3, 11	<i>Scandix pecten veneris</i> .....	3
<i>Erysimum cheiranthoides</i> .....	3, 22	<i>Scirpus acutus</i> .....	11, 23
<i>Euphorbia maculata</i> .....	11, 22	<i>Setellaria media</i> .....	9, 14
<i>Foeniculum vulgare</i> .....	11, 23	<i>Silybum marianum</i> .....	11, 23
<i>Galeopsis tetrahit</i> .....	3	<i>Sisymbrium altissimum</i> .....	22
<i>Glechoma hederacea</i> .....	22	<i>Solidago occidentalis</i> .....	11
<i>Helianthus annuus</i> .....	22	<i>Sonchus arvensis</i> .....	9
<i>Hydrocotyl proliferus</i> .....	11	" <i>asper</i> .....	23
" <i>rotundifolia</i> .....	14	" <i>oleraceus</i> .....	23
" <i>umbellata</i> .....	23	<i>Sophis</i> spp. ....	22
<i>Hypericum perforatum</i> .....	11	<i>Sparganium eurycarpum</i> .....	11, 22
<i>Hypochoeris radicata</i> .....	23	<i>Spergula arvensis</i> .....	3, 18
<i>Iva xanthifolia</i> .....	22	<i>Taraxacum officinale</i> .....	9, 11, 14, 22, 23
<i>Jussiaea californica</i> .....	11	<i>Thlaspi arvense</i> .....	3, 18, 22, 23
<i>Kochia scoparia</i> .....	22	<i>Tribulus terrestris</i> .....	22
<i>Lactuca pulchella</i> .....	23	<i>Trifolium repens</i> .....	11
" <i>scariola</i> .....	23	<i>Urtica</i> spp. ....	23
<i>Lamium amplexicaule</i> .....	22	<i>Verbena bracteata</i> .....	22
<i>Lappula echinata</i> .....	22	<i>Veronica hederifolia</i> .....	3
<i>Lepidium apetalum</i> .....	22	" spp. ....	22
" <i>virginicum</i> .....	22	<i>Xanthium canadense</i> .....	23
<i>Malva parviflora</i> .....	23	" <i>spinosum</i> .....	23
" <i>rotundifolia</i> .....	9, 22, 23	" spp. ....	22
<i>Medicago lupulina</i> .....	22, 23		

\* It is impossible to separate plants into distinct groups with respect to their reaction to growth regulators. Some of the plant species included here were tested as seedlings treated through the soil; others were sprayed as young or mature plants. Differences of opinion found in this table undoubtedly result from such differences in testing methods. This grouping represents interpretation by the writer of descriptions of experiments published by the workers cited. Materials used in the various tests reported include 2,4-dichlorophenoxyacetic acid and its sodium salt in this country, and 4-chloro-2-methyl phenoxyacetic acid and its sodium salt in Britain.

acid or its salts) solutions have been applied to weeds growing in the field. The weed species include several biennials and perennials, and the obser-

vations involve results of translocation as well as contact toxicity. Concentrations of 0.2 and 0.1 per cent. were used, and most of the plants were large and actively growing when treated. Seedlings have proved somewhat easier to kill; plants in the late-blossom and seed-forming stages, more difficult.

This listing of species susceptible and resistant to the action of 2,4-D is a useful index to the weeds that may be treated successfully. It is helpful also in indicating the possibility of selectivity in the control of weeds in crops.

### Discussion and summary

Selective killing of certain plant species from mixed plant populations is a common phenomenon, and selectivity is based upon many differences. Among these are differences in wettability, in exposure of essential plant parts such as meristems, in orientation and distribution of leaves, in depth and distribution of roots, and in chemical tolerance to certain toxic substances. This last type is probably the most promising in selective weed control because methods based on chemical tolerance depend largely upon composition of the herbicidal material and stage of growth of the plants—factors that can be regulated. Wettability, on the other hand, may be largely determined by plant characteristics or by weather conditions before and during treatment. Exposure as related to orientation and distribution of plant parts is also beyond control, being inherent in the crop or weed species involved.

Introduction of the dinitro compounds, the selective oils, and the growth-regulating compounds has greatly enlarged the field for selective control of weeds. New crops have been added to the list of resistant species, and more will be found in the future. Although increases in toxicity and reduction in costs may even yet be effected, greatest promise lies in the broadening of selectivity and in the discovery of new crops that submit to selective herbicidal action.

Among crop plants, the Cruciferae present one group that should receive attention.

The experimental killing of grass seedlings in wild mustard with an oil fraction should offer a good lead to follow. The beet and mangel crops are another important group, and composites of the lettuce type represent a third. The control of weedy grasses in these broad-leaved crops would be a real advance; and the use of oil fractions warrants trial, since oils seem particularly toxic to grasses.

The dinitro substituted phenols have received detailed study but further exploratory work with them, seeking new crop-weed specificities, seems justified. Some work on the chlorinated phenols indicates that pentachlorophenol and its salts deserve further consideration. Ammonium pentachlorophenate offers possibilities as a noncolored selective spray (7) and pentachlorophenol is useful as a fortifying agent in oils and oil emulsions.

The growth regulators offer another great opportunity for testing selectivity. Although broad-leaved plants seem most susceptible to their action

and grasses generally tolerant, results of testing already show a wide difference among the former group and some variation among the latter. It seems possible that further testing of these compounds in their various combinations may disclose new and useful specificities among weeds and crops.

Dozens of growth regulators have already been tried, and many more will undoubtedly be synthesized. Since most of these are acids in their parent form, each offers opportunity to form metallic salts, esters, amides, and the like; almost innumerable compounds can be synthesized for testing. The systematic study of the toxicity and selectivity of these compounds offers a great opportunity for practical and scientific application of biochemistry and plant physiology in the service of agriculture.

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# NOTE ON THE EFFECT OF UNILATERAL ILLUMINATION ON THE TRANSVERSE ELECTRICAL POLARITY IN THE AVENA COLEOPTILE<sup>1</sup>

A. R. SCHRANK

(WITH ONE FIGURE)

## Introduction

The effect of incandescent light on the electrical potentials of etiolated seedlings has been studied by only a few investigators. WALLER (7) maintained that etiolated seedlings gave little, if any, electrical response when illuminated. CLARK (1), however, has demonstrated changes in the longitudinal electrical polarity in intact etiolated *Avena* seedlings when they are uniformly illuminated by 600,000 meter-candle-seconds. Isolated *Avena* coleoptile sheaths when illuminated manifest electrical polarity changes similar to the intact plants (5).

Previous experiments have shown that *transverse* electrical polarities with the same orientation with respect to subsequent curvature are established in the *Avena* coleoptile by gravity (6) and mechanical stimulation (3, 4). The present experiments show the effect of unilateral illumination on the transverse electrical polarity in the apical region of the isolated coleoptile sheath.

## Materials and methods

*Avena sativa*, Victory Strain (C.I. 2020, obtained from the U. S. Department of Agriculture), was used in these experiments. The procedure for sprouting and growing the seedling has previously been described (3, 4, 6).

A 100-watt General Electric frosted mazda lamp was used as the light source. This lamp was housed in a box with the open side facing the coleoptile. The distance between the lamp and the coleoptile was 1 meter. This arrangement gave a light intensity of 16 foot-candles (measured with a Model 603 Weston Illumination Meter) at the coleoptile position. The temperature at the coleoptile position was not affected by the light because the air in the room was circulated.

The experiments were performed in an air-conditioned dark room under a neon light constructed out of ruby glass tubing. Electrical measurements were made with a du Bridge (2) vacuum tube voltmeter using isoelectric ( $\pm 0.25$  mv.) zinc-zinc sulphate electrodes. Glass tubes drawn to 1 millimeter in diameter were used as contacts with Shive's solution in tap water as the contact medium.

## Experimental

Coleoptile sheaths 25 millimeters long isolated from 30 millimeter ( $\pm 1$  mm.) plants were used in all experiments. These were placed in the holder and allowed to remain undisturbed in the apparatus for 1 hour before start-

<sup>1</sup> Supported by the University of Texas Research Institute.

ing the experiments. E.M.F. and temperature readings were taken for 10 minutes before the light was turned on. The light was then turned on and left on as indicated in figure 1. Curvature measurements were made by observing the position of the coleoptile tip on a horizontally placed ocular micrometer (4).

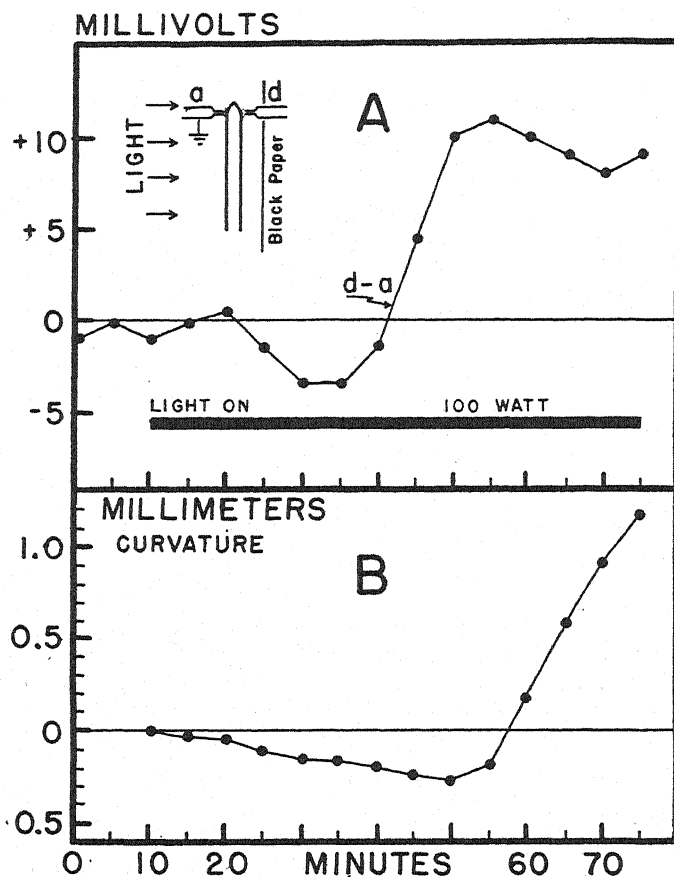


FIG. 1. Effect of continuous unilateral illumination on an isolated *Avena* coleoptile sheath. A. Changes in the transverse electrical polarity 0.5 millimeter below the apex. B. Phototropic curvature of the same sheath.

The curve  $d-a$  in figure 1, A, shows the changes in the transverse electrical polarity in an isolated coleoptile sheath 0.5 millimeter below the apex when illuminated unilaterally by a light intensity of 16 foot-candles at the position of the coleoptile. The curve as plotted shows the electrical variation of the contact labelled  $d$  with respect to the grounded contact  $a$ . The opposite sides of the coleoptile, as indicated by the E.M.F. curve for the first 10 minutes, are isopotential before illumination. After 10 minutes of illumination the contact region  $d$  (shaded side) becomes electronegative to



the contact region *a*. This first negative variation was observed in about 65 per cent. of the experiments performed. The shaded side then becomes electropositive to the lighted side. The average maximum electrical polarity in this series of experiments was 8.2 millivolts.

The curve in figure 1, B, shows the phototropic bending of the same coleoptile whose E.M.F. changes are shown in figure 1, A. This coleoptile shows a slight initial negative curvature (away from the light). About half of the plants that were used showed this curvature. The positive curvature (toward the light) in this experiment started 40 minutes after the light was turned on. It should be noted that the shaded side of the plant becomes electropositive to the lighted side before the bending toward the light begins. This was observed in all of the 18 experiments in this series.

All experiments were performed at 26° C. ( $\pm 1.0$ ) with a variation of not more than  $\pm 0.3^\circ$  C. during a single experiment.

A second series of experiments, in which the contacts were placed 2 millimeters below the apex, verified the facts shown by figure 1. It is of interest to note, however, that the first variation in E.M.F. (shaded side electronegative to the lighted side) which is shown in figure 1, A, was never observed with the contacts 2 millimeters below the apex.

### Discussion

The data from these preliminary experiments demonstrate two facts. First, continuous unilateral illumination establishes a transverse electrical polarity in the apical region of the isolated *Avena* coleoptile; the shaded side becomes electropositive to the lighted side. Secondly, this transverse electrical polarity is established before the bending toward the light begins. Previous experiments have shown that gravity (6) and mechanical stimulation (3, 4) cause the coleoptile to establish similar polarities. In all three cases the polarity has the same orientation with respect to the subsequent curvature. The electropositive side always becomes the convex side. Also, in each case, the electrical polarity is established before the curvature begins.

### Summary

The apical region of isolated coleoptile sheaths establishes a transverse electrical polarity when it is unilaterally illuminated. The shaded side becomes electropositive to the lighted side. This transverse electrical polarity is established before the phototropic curvature begins. The orientation of this polarity with respect to subsequent curvature is the same as established by gravity and mechanical stimulation.

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## BRIEF PAPERS

### THE EFFECTS OF AN INCREASE IN PRESSURE ON THE ACTION OF DIASTASE IN VITRO

H. C. EYSTER

To study the effects of an increase in pressure on the action of diastase, a pressure pump was acquired. It was one constructed on the tire-pump principle and operated in a similar manner. A mercury gauge was used to measure the increase in pressure of the atmosphere as it was forced into the flasks which contained the diastase mixtures. As soon as the desired pressure was attained, it was maintained by securely applying strong pinch clamps to the rubber tube outlets. Subsequent measurements with the mercury gauge showed that the pressure was being maintained.

The technique used here is the same as that described by EYSTER (1). The enzyme, diastase of malt Merck (medicinal, U.S.P. IX), was adsorbed upon particles of activated charcoal. The effectiveness of diastase as a hydrolytic agent for soluble starch was measured by determining the time required to digest a given amount of soluble starch past the last iodine staining stage. Dried Norit A kept in a desiccator was the type of activated charcoal used (1). The temperature was constantly checked and always kept at approximately 25° C. by the use of a water bath.

#### Data and results

In one series (A) of experiments, 50 ml. of 1 per cent. soluble starch, 45 ml. of distilled water, and 5 ml. of 1 per cent. diastase were mixed and 1 g. of Norit A promptly added. To test the influence of auxins, as well as the influence of an increase in pressure, indole-3-acetic acid was used in another series (B). With auxin, the mixtures had 50 ml. of 1 per cent. soluble starch, 40 ml. of distilled water, 5 ml. of a solution containing 500 p.p.m. of indole-3-acetic acid (25 p.p.m. in the final mixtures), 5 ml. of 1 per cent. diastase, and 1 g. of Norit A. Both series were performed in the dark. In each of the two series, three (controls) were subjected to atmospheric pressure, and three others were subjected to an additional 15 inches (Hg) of pressure. The data were as follows:

#### *Series A (without auxin)*

Controls at atmospheric pressure .....	Close to 360 minutes
At "Plus 15 inches Hg" pressure .....	" " 420 "

#### *Series B (with auxin)*

Controls at atmospheric pressure .....	Close to 300 minutes
At "Plus 15 inches Hg" pressure .....	" " 330 "

This experiment was repeated twice and the data were the same as in the original trial. These data indicate that an increase in pressure definitely retards diastase activity when it is associated with charcoal. Under

increased pressure the diastase appears to become more securely bound to the charcoal surfaces. It has been previously determined by the author (1, 2) that auxins release diastase from charcoal surfaces to which diastase had become adsorbed; it is thus natural that the presence of auxin should reduce the total time for digestion of starch past the last iodine staining stage or in other words to increase the rate of digestion by diastase. In the presence of auxin, just as in the absence of auxin, an increase in pressure retards digestion by diastase.

The effect of an increase in pressure and the effect of auxins were determined, also, for isolated systems of diastase. In the series (C) without auxin, 50 ml. of 1 per cent. soluble starch, 48 ml. of distilled water, and 2 ml. of 1 per cent. diastase were used. Fifty ml. of 1 per cent. soluble starch, 10 ml. of a solution containing 500 p.p.m. of indole-3-acetic acid, 38 ml. of distilled water, and 2 ml. of 1 per cent. diastase were used in the series (D) of tests with auxin. The final concentration of auxin in the mixtures was 50 p.p.m. Three mixtures were tested in each case. The results were as follows:

*Series C* (without auxin)

Controls at atmospheric pressure .....	45 minutes
At "Plus 15 inches Hg" pressure .....	36 minutes

*Series D* (with auxin)

Controls at atmospheric pressure .....	60 minutes
At "Plus 15 inches Hg" pressure .....	57 minutes

Here too the experiment was repeated twice, and the data were very closely the same in absolute value and exactly the same in relative or comparative value as for the original trial. These data indicate that an increase in pressure definitely accelerates diastase action when the diastase is in isolated systems; i.e., is not associated with an adsorbing agent like charcoal. Auxin in series D retards the action of diastase and the accelerating effect of an increase in pressure on auxin mixtures is questionable. The difference in the digestive rates with auxin is really not of sufficient magnitude to be significant; but the controls always were slower, and the time required to digest a given amount of soluble starch past the last iodine staining stage was always greater.

The retarding effects of an increase in pressure on the digestion brought about by diastase associated with charcoal may present a clue as to the reason for positive hydrotropism in roots. It is well known that roots generally grow, in a soil where there is a moisture gradient, toward a higher moisture content. It is known that roots are very sensitive to extremely minute differentials of soil moisture. With the slightest difference in the moisture content of the soil on the two sides of a very slender root tip the root tip normally grows toward the side where the moisture content is greater. This would mean that the growth is less on the more moist side and greater on the less moist side. The cells on the more moist side would

be expected to have a greater water content, and hence a higher turgor pressure. The cells on the less moist side would be expected to have less water, and hence a lower turgor pressure. Cellular enzymes are associated with cellular colloids, perhaps much like the diastase in this paper was adsorbed upon activated charcoal. Since it has been found that an increase in pressure retards the action of diastase which is associated with activated charcoal, the lower turgor pressure on the drier side of the root or rootlets would then nicely explain the greater growth on that side, a growth away from the drier soil and toward the greater amount of soil moisture.

### Summary

An increase in pressure accelerates the digestive rate of diastase in isolated mixtures but retards the digestive rate of diastase associated with activated charcoal. The effect of turgor pressure on the activity of cellular enzymes associated with cellular colloids may explain the reaction resulting in the normal positive hydrotropism of roots. The lower turgor pressure on the drier side of a root would nicely account for the greater growth on that side, inasmuch as it has been found that the action of diastase associated with activated charcoal is inversely proportional to the pressure.

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1. EYSTER, H. C. Auxin action. *Science* **97**: 358-359. 1943.
2. ———. Effect of auxins on the action of diastase in vitro. *Plant Physiol.* **21**: 68-74. 1946.

RIDGES AND SECTORS INDUCED IN OLIVE FRUITS BY  
FUMIGATION WITH HYDROCYANIC ACID

WALTON B. SINCLAIR AND DAVID L. LINDGREN

In a previous paper (1), the authors have shown that the fumigation of citrus trees with hydrocyanic acid (HCN) for pest control, at a time of the year when the fruit buds are in a certain stage of development, produced ridges and sectors in the outer portion of the peel (flavedo) of the fruit. In the navel orange, Valencia orange and grapefruit varieties, fumigation of trees in February produced the greatest amount of ridged fruits, but in some years extreme ridging of the fruits occurred on trees fumigated the latter part of January. In the lemon variety, trees fumigated in February produced the highest percentages of fruits with ridges and sectors, but ex-



FIG. 1. Ridges on olive fruits (Manzanillo variety) caused by fumigating the tree when the fruit was in the bud stage of development.

tensive field observations have shown that fruits of lemon trees fumigated from late January to April, inclusive, were severely affected. Variation in the time and rate of bud development of citrus trees in different groves and in different years indicated a definite variation in the time of the year in which ridging was produced in the fruit by fumigation. The development of ridges and sectors on citrus fruits depended mainly upon the stage of development of the buds at the time the trees were fumigated.

Since these results on citrus were published, Mr. A. F. KIRKPATRICK, working on the development of a program for the control of parlatoria scale (*Parlatoria oleae* Colvee) on olive trees by HCN fumigation, called to the



authors' attention a similar phenomenon on olive fruits (fig. 1). As in citrus, the fumigation of olive trees in the spring at a particular and definite stage in the development of the bud resulted in ridges and sectors on the fruit. The occurrence of this phenomenon on a fruit other than citrus and under similar experimental conditions substantiates the published results of the authors (1). It is highly suggestive that this phenomenon may demonstrate a general physiological effect of HCN if experiments were performed on a sufficient number of fruit varieties.

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#### LITERATURE CITED

1. SINCLAIR, WALTON B., and LINDGREN, DAVID L. Ridges and sectors induced in the rind of citrus fruits by fumigation with hydrocyanic acid. *Plant Physiol.* **18**: 99-106. 1943.

## NOTES

**Election of Officers.**—The following have been elected as new officers of the American Society of Plant Physiologists: President, B. M. DUGGAR; Vice-president, S. F. TRELEASE; Executive Committee, H. A. SPOEHR; Editorial Board, E. J. KRAUS. The terms of office for the president and vice-president are for one year. Members of the executive committee and editorial board are elected for three-year terms.

**Meeting of Western Section.**—The Western Section of the AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS held a joint meeting with the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE and affiliated societies at Reno, Nevada, June 18–20. Eighteen papers were presented including a symposium on plant growth relations in saline soils at a biologists' dinner on June 19. A joint session was held on the morning of June 19 with the WESTERN SOCIETY OF SOIL SCIENCE, the AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE, and the BOTANICAL SOCIETY OF AMERICA. The morning session of June 20 was a joint meeting of the AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS with the AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE. A business meeting was held on June 19. The officers of the Western Section of the A.S.P.P. are: F. W. WENT, Chairman, C. H. WADLEIGH, Vice-Chairman, ORLIN BIDDULPH, Secretary and Chairman of the program committee. DOCTORS WENT and BIDDULPH are also representatives on the Council of the PACIFIC DIVISION of the A.A.A.S.

**Frances Louise Long.**—THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS mourns the passing of DR. LONG who died at Santa Barbara, California, on March 17, 1946.

DR. LONG spent most of her life with workers in ecology but was drawn constantly toward physiological problems and the phases of morphology involved in these problems. Her work was only partially expressed in her publications, for the work of many of the later years of her life was given largely to the laboratory and field work involved in the attempts to understand the adaptation and origin of plant species. This work was left without final interpretation by the death of DR. CLEMENTS and the death of DR. LONG a few months later. Her experimental work was done largely at Tucson, at the Alpine Laboratory near Pike's Peak, and at the Coastal Laboratory at Santa Barbara. She also travelled widely.

DR. LONG was born at Madison, Nebraska, September 7, 1885. She received her B.A. and B.Sc. degrees from the University of Nebraska in 1906. In 1914 she received a M.A. degree from the University of Minnesota and in 1917 a Ph.D. degree from the same institution. She was assistant instructor in botany at the University of Minnesota from 1912 to 1917. In 1917 she began ecological research for the Carnegie Institution of Washington where she served under the direction of DR. F. E. CLEMENTS until he retired in 1941. However, she did not stop work at that time but continued until a few weeks before her death.

In 1915 a study of the effect of six years of desiccation and starvation on *Ferocactus* was published by MACDOUGAL, LONG, and BROWN. In 1919 DR. LONG published "The Quantitative Determination of Photosynthetic Activities in Plants." Studies carried on chiefly on *Asclepias* were published by HALL and LONG under the title "Rubber Content of North American Plants" in 1921. Studies of experimental pollination as it dealt with insect-flower relations were continued from 1918 to 1925. In 1923 CLEMENTS and LONG published "Experimental Pollination, an Outline of the Ecology of Flowers and Insects." Other studies included latex and lactiverous plants, translocation and storage in autumn, stomatal behavior of giant cactus, calorimetry and adaptation and origin in plants. In 1927 MACDOUGAL and LONG published "Characters of Cells Attaining Great Age." In 1929 DR. LONG published "Stomata Which Show Functional Movement for a Century," in 1934 "Application of Calorimetric Methods to Ecological Research" and "The Method of Collodion Films for Stomata." CLEMENTS and LONG published "Factors in Elongation and Expansion under Reduced Light Intensity" in 1934 and in 1935 "Further Studies of Elongation and Expansion in *Helianthus* Phytometers."

DR. LONG was a member of the American Association for the Advancement of Science, the Ecological Society of America, the Botanical Society of America, the American Society of Plant Physiologists, and of Sigma Xi. She also took an active part in local scientific and social organizations.

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**Luther Burbank, A Victim of Hero Worship.**—W. L. HOWARD. Volume IX of *Chronica Botanica*, 1945. *Chronica Botanica Co.*, Waltham 54, Massachusetts and *G. E. Stechert and Co.*, 31 E. Tenth St., New York City. 506 pages. \$3.75.

Some twelve years ago DR. HOWARD undertook the preparation of an authentic list of all fruits, flowers, vegetables, and tree fruits which the late LUTHER BURBANK introduced during the fifty years of his working life. The task was difficult because BURBANK kept no systematic record of what he produced and his advertising literature was widely scattered. The author personally examined libraries and private collections throughout the United States. The value of many of BURBANK's productions was determined by contacting scientists all over the world. The book contains considerable new biographical information about BURBANK which sheds light on many puzzling questions.

The leading events of BURBANK's life are discussed objectively, but fairly, frankly and without embellishment. The author gives a true and complete picture of BURBANK about whom there has been so much controversy, describing his labors of 50 years in improvement of economic plants and listing over 800 new varieties of fruits, flowers and vegetables, many of them of permanent value, which BURBANK produced. The book is an important contribution as the biography of BURBANK and as history of plant

breeding. The style is dignified yet interesting, the facts having obviously been presented with the impartiality and understanding of a distinguished expert. Contents: The background. The man. The nurseryman. The scientist. The egoist. The mentor of youth. The unfortunate. The pariah (of scientists). The disappointed. The world character. The individualist. Ethics. Religion. Foray into science—the CARNEGIE GRANT. Admirers. Detractors. BURBANK's place in the hall of fame. Summary of BURBANK's productions. Aftermath. The BURBANK family.

**Phosphates and Superphosphates, Second Edition.**—A. N. GRAY. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 416 pages. \$7.00.

This second edition is greatly enlarged from the first, originally published in 1930, and furnishes a more complete coverage of the subjects. The author is secretary of the International Superphosphate Manufacturers Association and is well qualified to prepare a world-wide treatise on phosphates. Included are chapters on the world deposits and reserves of phosphate rock and other phosphate minerals, and on their production and consumption in agriculture and commerce. Other chapters cover the methods and practices in the production of normal and concentrated superphosphates, basic slag, calcined phosphate, and other phosphate bearing products used in world agriculture, their cost of production, and their utilization and consumption.

The new edition is well illustrated with 38 pictures, mostly of phosphate rock mining methods and machinery for superphosphate manufacture. Many tables and analytical data appear throughout the text and in addition there are 155 statistical tables on phosphate rock and superphosphate world trade. Maps of the phosphate rock deposits of the world are on the inside cover. Manufacturers of superphosphate will find this a well-worth-while book, valuable not only to the technical and production staff but as a reference work for executives in connection with the place of phosphates in world economy.

**The Chemistry of the Carbon Compounds.**—Volume III, The Aromatic Compounds. VICTOR VON RICHTER, and RICHARD ANSCHUTZ, Third English edition based on the Twelfth German Edition, translated by A. J. MEE. Elsevier Publishing Co., 215 Fourth Avenue, New York 3, N. Y. 794 pages. \$15.00.

Biologists will welcome volume III of the new English edition of this well known treatise. The content has been brought up to date and citations are given to original journals and authors instead of to abstracts in the *Chemisches Zentralblatt*. The new arrangement of contents covers two major divisions; I. Mononuclear Aromatic Compounds (Benzene Derivatives) and II. Multinuclear Compounds (Phenyl-benzenes, Polyphenyl-

aliphatic-Hydrocarbons, and Condensed Aromatic Ring Systems). The volume carries a complete and cross-referenced index of eighty pages.

**Journal of Polymer Science.**—Edited by P. M. DOTY, R. HOUWINK, H. MARK, and C. C. PRICE. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 640 pages. Subscription price \$8.50 per year.

Numbers one and two, Volume I (147 pages) of this new bimonthly journal for January and March, 1946, are now available. The new journal is an outgrowth of, and a successor to, the Polymer Bulletin which has ceased to appear. The new journal is devoted to the advancement of fundamental knowledge of the physics and chemistry of polymers, offering to scientific workers in this field an opportunity to report experimental and theoretical contributions through a centralized medium. It will be the editorial policy to encourage freedom of discussion and to provide prompt publication. The Journal will publish original papers, review articles, brief communications to the editor, and book reviews. It will be international in scope. To this end, a number of leading scientists in the field have been invited to form an Advisory Board, which represents all the important centers of polymer research in the United States and abroad, as well as scientific societies and their divisions whose fields of activity are within the scope of the journal. The wide ramifications of polymer chemistry into physics, physical chemistry, organic chemistry, colloid chemistry, and biology were taken into account in forming the Advisory Board. All papers will be published in English. The publishers are willing to provide further information and supply sample copies on request.

**Physical Methods of Organic Chemistry.**—Volume II, edited by ARNOLD WEISSBERGER. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 630 pages. \$8.50.

The second volume extends the description of volume I on physical methods of importance to organic chemists and biologists. The two volumes of this monograph endeavor to assemble the hitherto widely scattered literature on physical techniques with a view to relieving investigators of the time-consuming burden of bibliographical search. The volume comprises tested methods along with a discussion of theoretical considerations necessary for understanding and handling of various procedures. Attention is given information required for critical evaluation of experimental results. Volume II contains sections on Spectroscopy, Colorimetry, Polarimetry, Dipole Moments, Conductometry, Polarography, Magnetic Susceptibility, Radioactivity, Mass Spectroscopy and concludes with a fifty-two page index to volumes I and II.

**Scientific Progress in the Field of Rubber and Synthetic Elastomers.**—Volume II of Advances in Colloid Science edited by H. MARK and Z. S.

WHITBY. Interscience Publishers, Inc., New York 3, N. Y. 453 pages. \$7.00.

This monograph endeavors to collate and evaluate in a single volume the extensive literature on natural and synthetic, elastic colloid, high molecular weight rubbers and elastomers. An excellent introductory discussion covers the rubber state and related nomenclature, natural and synthetic rubbers and their compounding. Subsequent sections, all written by specialists, cover transition effects in rubber and other high polymers, crystallization phenomena, results of studies by X-ray diffraction methods, thermodynamic data on rubber solutions and gels, viscosity measurements, kinetic theory of rubber elasticity, vulcanization and rubber photogels. The volume carries separate author and subject indexes.

**An Introduction to Botany.**—Second Edition. Edited by ARTHUR W. HAUPT. McGraw-Hill Book Company, Inc. New York and London. 425 pages. \$3.50.

The new second edition of this well known text covers clearly and concisely the fundamental facts and principles relating to the structure, functions, life relations, and evolution of plants. Designed for a one-semester course in botany, the book does not attempt to present an exhaustive treatment of the subject, but to furnish a foundation upon which more advanced studies may be based. Emphasis is placed upon morphology and considerable attention is given to the principal physiological processes. The book has been entirely rewritten, and significant new material has been added. A feature of the second edition is the list of visual aids—motion pictures and film strips—correlated with the material in the text.



### PREPARATION OF MANUSCRIPTS FOR PUBLICATION IN PLANT PHYSIOLOGY

The following suggestions are made to authors writing papers for this journal in order to minimize revisions and editorial corrections. Though many points may appear arbitrary or trivial, attention to details by authors is necessary to secure uniformity of format and style. The membership dues and subscription fees go almost entirely to publication costs, as all of our editors give their service without financial recompense. Hence the cooperation of authors in preparation of manuscripts conserves our funds and permits greater liberality as to length and number of papers published.

1. In the preparation of typescripts, authors are requested to examine a printed copy of the journal of Plant Physiology to note the position of headings, general organization, and the methods of citing literature particularly, in order to bring the script into conformity with the general architecture of published papers.

2. Leave a 3-inch margin at the top of the first page, liberal side margins for editorial marks, and double spacing of lines so that there is space for any required editorial changes.

3. Avoid footnotes as far as possible. They are expensive. If you do use them, number them with Arabic numerals.

4. Place your name and figure number on the back of each figure. Do not put legends into or on the figure, either front or back. Type all legends, properly numbered to correspond to the figures, on a separate sheet of your manuscript. Graphs and drawings should be inked with fairly heavy lines to insure clarity of text figures after reduction.

5. Leave headings unscored, scientific names without underlining, etc. The editors will take care of all of these matters in accordance with the practice of the journal. Generic names used alone are not italicized. In general, do not underscore any lines.

6. Place each table on a separate page; do not crowd too much material into a single table. Note size of printed page (width and length,  $4\frac{1}{2} \times 7$  in.) and gauge tables accordingly, leaving plenty of space in margins and in the interior for editorial marks. If at all possible, arrange tabular data horizontally on the page. Use horizontal lines at top and bottom and in column headings, but not in interior of tables. Use vertical lines between columns. Spaces (leads) are usually used in the final printed tables to separate sets of data. For footnotes to tables use the asterisk, dagger, double dagger, and section. Tabular material should be used sparingly. It costs over \$12.00 per page. Authors will be billed for the cost of tabular data and figures in excess of 25 per cent. of the total paper.

7. Place acknowledgments at the end of the paper. The name of your institution and its address should also be given at the end of the manuscript.

8. Use separate pages for the literature cited. Give complete citations, author, title, journal name, volume number, inclusive pages, and year. (See any literature list in the journal for examples.) Punctuate according to the

examples, and leave no citations incomplete. Write out names of states in full. Note proper abbreviations of journals.

An error frequently noted by our editors in manuscripts with long bibliographies is the failure to mention in the text all citations listed. Authors can assist in this matter by checking off the citations in the bibliography as they encounter reference to them in the body of the text in proof-reading copy prior to submission.

9. Limit the size of all pages, drawings, tables, and photographs to standard  $8\frac{1}{2} \times 11$  inches, or less.

10. Use mathematical formulae only when necessary, and be conservative in the use of space.

11. Authors will be billed for alterations in the galley proofs involving excessive changes from manuscript copy.

12. Send original copy of manuscript to editor, W. F. Loehwing, University of Iowa, Iowa City, Iowa. Retain a carbon copy to insure against loss, and to consult in case of need. We solicit your cooperation in these matters to relieve the editors and printers of some of their most frequent and costly difficulties.



# PLANT PHYSIOLOGY

OCTOBER, 1946

## THE CASE FOR GREATER COOPERATION AMONG THE PLANT SCIENCE SOCIETIES<sup>1</sup>

BERNARD S. MEYER

Dispersive tendencies have long been at work among the biological organizations in this country, with the result that there are today at least thirty-five different biological societies of national scope. The aggregate membership of these organizations is between 30,000 and 40,000. Yet with only two or three exceptions, none of these societies has a membership of more than 2,000, and most of them are considerably smaller. Each of these organizations has its president, vice-president, secretary, and other officers. Many of them publish their own journals. With only one or two exceptions, all of these societies are run on a strictly amateur basis, the often arduous duties of secretaries, treasurers, editors, and business managers being performed gratuitously by those members who have been elected or appointed to such positions by the combined efforts of their friends and enemies.

The organizational division and subdivision of the biological fraternity is a matter of sincere concern to many thoughtful plant and animal scientists today. In many ways the lack of centralized organizations of biologists has operated against them, and will continue to do so. At no time has this fundamental weakness in the body politic of biologists become more glaringly apparent than during the war. The lack of a coherent and integrated focal organization contributed to the comparatively ineffective utilization of the specialized and often unique talents of biological scientists in the war efforts.

Appalling examples of the misuse of biological manpower during the war can be cited. One of the most brilliant of the younger plant cytogeneticists in this country died on the beaches of Salerno. Another promising young botanist perished in the torpedoing of a Japanese prison ship off Mindanao. And these are not the only biologists whose contributions have been lost forever. Such men as these should never have been in the army at all. And of all branches of the army least of all should they have been in the infantry. Not even by their own choice should they have been allowed in the forefront of the fighting, not for their own sake, but for the sake of

<sup>1</sup> Delivered as the address of the retiring president of the American Society of Plant Physiologists for 1943-44 at St. Louis, Missouri, March 29, 1946.

the national welfare. There were dozens of more important jobs for them, both in and out of the armed services.

Less tragic, but still serious when measured in the currency of social loss, because of the multiplicity of cases, was the waste of biological manpower by the armed services under the guise of making proper use of it. Many a capable botanist or zoologist was assigned to the medical corps, for example, only to find himself relegated to some trivial non-biological occupation, or to the performance of some routine biological test at about the technological level of a sophomore in pre-medical school.

Nor was the misuse, or failure to use biological manpower where needed, confined to the armed forces. The situation in civilian life was not notably better. Many new and unexpected research problems arose out of the necessities and exigencies of global war. Many of these problems were either biological or they had important biological aspects. But biological manpower was never effectively mobilized for work on such problems. Sometimes the right man fell into the right job, but the contrary was more often true. Biologists were overlooked, bypassed, or given only secondary responsibilities in scientific endeavors for which they were fully competent to assume leadership.

One glaring example of the failure to direct botanical manpower into the proper channels was described by Dr. Griggs in his address at the Cleveland meeting several years ago. Over half a million dollars were reportedly spent on a certain fungous project which could have been done by competent mycologists for a small fraction of the sum. The agency in charge did not know where to turn for assistance; indeed, there is no evidence that they were aware of the existence of mycologists.

Some miscasting of actors is inevitable in the hastily plotted drama of total war. But the non-use or misuse of biological manpower was so widespread, so glaring, and at such a variance with sound public policy, that it should be a matter of serious concern to all plant and animal scientists.

No one person nor group is at fault for these costly and tragic mistakes in the utilization of scientific manpower. Some of the fault lies near home. Some of it even lies within this room, with you, with me, and with all other biologists. We failed ourselves and our colleagues when we neglected to provide a mechanism by which the possible services of biologists could be brought into better focus in the minds of governmental agencies, both military and civilian. If one or two strong, centralized bodies of biologists had been in existence, and had properly exerted their influence, it is certain that fewer mistakes in the allocation of biological manpower would have been made.

The war has now receded into history, but the duration is proving to be much longer than the war. We are now in a difficult period of postwar readjustment which is a part of the world-wide political, economic, and scientific disequilibrium. We—as plant scientists—were not properly organized for war, but it is not too late to organize for peace. Other problems

in which plant and animal scientists have a legitimate interest and about which they will wish to speak will constantly be coming to the fore. Without spokesmen, however, whose voices can ring with the authoritative support of all biologists, or at least of substantial groups of biologists, the scientists' chances of being heard will be very slim.

The bill for the establishment of a National Science Foundation now emerging from a congressional committee represents the most outstanding current project in which scientists have a vital stake. Polls indicate that an overwhelming majority of scientists is in favor of such legislation. After a somewhat disconcerting period of diversity of opinion and controversy, the sentiment of most scientists seems finally to be crystallizing behind S 1850, which embodies most of the best features of several previous bills.

The writing of a bill is only the first step in legislation and by no means insures its passage. No version of a bill for a National Science Foundation has ever been considered on the floor of the Senate, and the House is scarcely aware of the existence of such proposed legislation.<sup>2</sup> Neither is there any great public clamor in support of such a bill. A rising sentiment for economy may also prejudice Congress against new ways of spending money. Any hope for the passage of such legislation may therefore yet depend on the mobilization of the maximum possible influence of scientists themselves in its support. Strongly centralized bodies of scientists (organizations conspicuously lacking among biologists), are necessary if the most effective representations in support of the passage of such legislation are to be made.

If such a bill is passed, and a National Science Foundation becomes a reality, there will be the problem of continuing relations with this organization. The necessary liaison between individual biologists or small groups of biologists in specialized fields and such a national foundation could best be accomplished through the intermediary of a centralized coordinating organization.

Of all the major groups of scientists, none has lagged so far behind in the development of organizational coherence and esprit de corps as the biologists. The chemists are strongly entrenched in the bosom of their powerful American Chemical Society. The physicists have their American Institute of Physics. The psychologists have recently stifled secessionist movements within their ranks by the formation of a new centralizing organization. The constitution for an American Geological Institute has recently been drawn up by representatives of eleven geological societies, and in all probability such an Institute will soon exist in reality as well as on paper.

Attempts to bring the biological sciences into closer organizational communion have not been entirely lacking. The Union of American Biological Societies, designed to accomplish this aim, was organized in 1923. Although the originally expressed purposes of the association were much broader, in

<sup>2</sup> Bill S 1850 was subsequently passed by the Senate, but the House committee to which it was referred failed to report it out. This, in effect, killed all such legislation for the recent session of Congress.



actual operation its activity has been largely confined to the sponsorship of the journal, *Biological Abstracts*. Membership of this organization is by societies, thirty-two of which now belong to the Union. The American Biological Society, whose objectives in general are similar to those of the Union of American Biological Societies, is a comparative newcomer to the biology landscape, having been started in 1941. Membership in the American Biological Society is by individuals, however, and not by societies. A move is now on foot to merge these two societies into a single stronger and more effective organization.

There are many proponents of some sort of a plan for bringing all species of biologists, either directly or indirectly, into one large "supersociety." Some move in this direction seems not only desirable, but almost imperative. Theoretically, this might be accomplished in several ways: one would be to expand some existing society into a position of leadership. A second would be to enlist every individual biologist into a new society which would be organized for the explicit purpose of representing the common interests of biologists. A third would be to form a federation of existing societies implemented with a central coordinating board or council and a full-time executive officer who could adequately and aggressively represent all the life sciences.

For various reasons the last-mentioned plan for a league of biological societies seems the most feasible one for strengthening the professional position of biologists. The framework of such a federation is already in existence in the Union of American Biological Societies. In the past the organization of this society has been too loose, and its existence too remote from the consciousness of individual plant and animal scientists for it to serve as an effective coordinating agency for biologists. But with a strengthening of its joints, a re-tooling of its objectives, and centralization of its efforts, the Union might conceivably be converted into an agency suitable to represent and to command respect for all of biology.

The proposal to unite all biologists under a single banner has definite merits and advantages. One of the most obvious advantages is the mobilization of the greatest possible numerical strength behind a central organization which would result. There are also difficulties and disadvantages to any project attempting to include scientists of every biological denomination. One difficulty is to persuade a sufficient number of biological societies to subscribe to an effective plan for cooperation. There are more than thirty such societies, and biologists, individually and collectively, are notorious individualists. Most biological societies have, in the past, maintained their affiliation with the Union of American Biological Societies, but it must be remembered that they have been asked for little more than moral support. Because an effective union must make heavy demands of time, money, and energy, the reluctance of individual societies to join or to maintain membership may rise steeply.

Another difficulty is the great diversity of interests that would be en-

compassed by such an organization. A union of all biological societies embraces a diversity of interest, although not an aggregate membership, so great as that represented by all of chemistry, all of physics, plus a sizeable segment of the engineering sciences. The greater the number of societies which affiliate through any sort of a central organization, the greater the diversity of interests represented. The diversity may prove an internal source of weakness and friction and may make adequate representation of all interests difficult. In particular, it seems that plant scientists might legitimately raise the question of whether their welfare could be best served by incorporation into any kind of an association in which they will be greatly outnumbered by animal scientists.

I am not attempting to discourage or disparage any efforts to federate all biological societies into a strong league for their mutual benefit, but I am attempting to look at the problem realistically. Certainly, such a plan is one possible solution to the problem under discussion, and it may be the best one. But in view of certain disadvantages of the project for a comprehensive biological union, and also in view of the fact that plans for an effective organization of this kind are in the embryonic stage, certain alternative proposals should perhaps also be given consideration.

Although certain great unifying principles run through all of biology, it does not necessarily follow that biology is *de facto* to be considered a unit science. Similar great principles run through all of physics and chemistry, though these two sciences are invariably recognized as separate and autonomous fields of inquiry. The term "biology" is taxonomically more nearly on a par with the term "physical sciences" than with the narrower designations "physics" and "chemistry."

There is a natural line of cleavage between the plant sciences and the animal sciences. Latterly, it has become the fashion in some circles to ignore the obvious dichotomy of interest, or to pretend that it does not exist. The demarkation between these two groups of biological sciences, however, is of the same order of magnitude as that between physics and chemistry, and to overlook it is to be blind to the facts of life. Of course there are overlapping areas, most of which represent important fields of scientific inquiry, but the same is true, and to relatively the same degree, of physics and chemistry.

An alternative suggestion would therefore seem to be that a Union of Plant Science Societies might be a more appropriate standard around which plant scientists could rally, than a federation of all biological societies. Organization of such a union would not necessarily exclude subsequent amalgamations with other biologists. There are only about ten national societies devoted exclusively to plant science, and I include in this group those in the applied fields such as horticulture, agronomy and forestry, as well as the more narrowly botanical groups. The chance of persuading such a group of societies to cooperate effectively through some sort of a super-organization appears more hopeful than the chance of inducing the thirty-

odd biological societies to fall into step with some similar proposal. The attainment of greater cooperation between the applied and theoretical plant sciences, which should be a by-product of such a cooperative project, would in itself be a worthy objective.

No plan should be followed for either a union of plant science societies or a union of all biological societies which will disturb the structural organization of existing societies. Most of the present societies have not only a long and honorable tradition, but also the established loyalties of their members. Any scheme which would attempt to disrupt these elements of the *status quo* would justifiably meet vigorous opposition.

The history of the American Institute of Physics is a pertinent object lesson for all botanists and zoologists. In 1931 the physicists of this country found themselves in a situation similar to that in which the biologists find themselves today. At that time the five major physics societies banded together to organize the American Institute of Physics. The organization has since acted as the spearhead for many kinds of cooperative activities among physicists. Membership in this Institute is by societies, not by individuals, but the member societies do not relinquish to the Institute their autonomy, title to their journals, control of meetings, maintenance of membership standards, or the responsibilities of their officers. The Institute is managed by a governing board to which three members are elected from each of the five constituent societies.

The activities of this organization have been manifold and only a few of the more important can be mentioned. The Institute assumed an important advisory rôle regarding the relation of physics and physicists to the war effort. It conducts a placement service for physicists. It considers questions of the establishment and maintenance of professional standards. Through the more dignified channels of publicity, it seeks to call attention to the achievements of physics. It cultivates closer and more understanding relations between physics and industry.

One of the major activities of the Institute has been supervising the publication of journals for the member societies, as well as publishing several journals in its own right. Major savings in publication costs have been achieved. Under this form of administration the constituent societies have paid out of dues, over a nine-year period, only 11 per cent. of the cost of publishing their journals. In addition, editors of the various journals have been relieved of many of the routine problems of publication.

Establishment of the Institute has assisted the member societies to augment their membership lists; increase the circulation of their journals, and advance their prestige in academic and industrial circles.

The Institute draws its financial support from a relatively small annual allowance from each of the member societies, profits of its journals and advertising therein, contributions of industrial and educational organizations, and grants from foundations.

Whether or not one is thinking of a plant science institute, an animal

science institute, or a federation of all biological societies, the American Institute of Physics provides a suggestive blueprint. Advantages and methods of financing such as those which the physicists have obtained might reasonably be expected to accrue to any sizeable group of biological societies pooling their common interests along lines somewhat similar to the American Institute of Physics.

Time permits only a sketchy presentation of the entire topic. But perhaps some of the verbal seed I have tried to sow will fall on receptive mental soils. The time for action in this matter will never be riper than the present. Only two courses are open: one is for us as individuals and as an organization to strive for a more centralized representation of our own and related sciences. The other, and unfortunately easier, course is to fall into a do-nothing or even a defeatist attitude. Each of us may say, "I am only one man. What can I do?" Or we may say, "This is only a small society. What can it accomplish?" Or we may even say, "Suppose we did have some sort of a union of biological societies or plant science societies, what could it do? The constituency of even such a group would be relatively so small in proportion to the enormous population of the country—could it really make its influence felt?"

Such sentiments remind me of an episode of my undergraduate days. There used to be, at Ohio State University, an annual fracas called the "cane rush," which took place every autumn between the freshmen and sophomores. The usually large horde of freshmen lined up at one end of a football field, and a much smaller group of sophomores at the other. The object was to carry a cane, initially in the sophomores' possession, to the opponents' goal posts. At a signal each group charged from its end of the field toward its opponents. The freshmen, by sheer weight of numbers, invariably won, despite any deception or artifice practiced by the sophomores. The inevitable outcome was not surprising as there were usually four or five freshmen to dispose of each sophomore. One year, however, the result was different. When the teams lined up, the sophomores did not assume their customary dispersed formation, but instead, arranged themselves into a wedge-shaped phalanx. Each man in the outermost row had his arms firmly linked with those of each of the men next to him. Within the outer row were other rows of men similarly linked together. Somewhere within this living wedge was the cane. Slowly but irresistibly the massive human triangle crawled down the field, clawed at by a surging horde of freshmen. But all attempts to dismember the phalanx failed. Some freshmen even took flying leaps on top of the phalanx—and promptly disappeared. Occasionally a man was peeled off the outer row of sophomores, but the gaps were always closed. When the final gun sounded the sophomores were across the freshman goal line with the cane still in their possession.

DEPARTMENT OF BOTANY  
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## THE COMING OF AGE OF THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS<sup>1</sup>

H. A. SPOHR

March 25, 1924, was the official birthday of our society. Owing to the exigencies of the war we were prevented from observing the attainment of its majority. As I had the honor of being president of the society during the twenty-first year of its existence, I feel that it may not be out of place to cast a retrospective glance over the youthful years of our society's life and to examine briefly what may lie ahead in its years of manhood. In venturing upon this theme let me stress that this is in no sense an official historical account; the opinions to be expressed are based upon my own impressions and should not be construed as representing a policy, or trend in policy, of the American Society of Plant Physiologists.

The birth of our society was not accomplished without some rather sharp birth pains. Yet, it is a kind provision of Nature that the throes of birth are soon forgotten in the joy of witnessing the young hopeful's growth and in the anxiety for its welfare. It has been a real satisfaction for all of us to have had a part in this experience. Sharing in this development has done much to foster the feeling of parental pride and cooperation which is characteristic of this group. A little more than a year after its birth we had the thrill of seeing our youngster cut his first tooth in the form of Volume I, Number 1, of our journal, PLANT PHYSIOLOGY. Since then we have had the gratification of seeing this journal attain its place among the foremost scientific publications of the world and become our most prized possession.

The fine things in this world are usually accomplished by men who do more than their share. This is eminently true of those who had the responsibility of editing our journal, particularly CHARLES A. SHULL and the succession of secretary-treasurers, to whom the destiny of our hopeful venture was largely entrusted. It is but fitting that at this juncture in the life of our society we express to these loyal guardians our continued gratitude and devotion.

Our membership has shown a gratifying and wholesome growth. The first list of members, published May 10, 1924, carried 76 names. Today we have 623 members. There are some advantages in a relatively small society for the purposes to which we are devoted. The intimacy, fraternal feeling, and spirit of cooperation engendered by a group in which the members are well acquainted have many advantages. We still have some missionary work to do so that those plant physiologists outside of our ranks and scientists in related fields may also enjoy the advantages which are ours through membership in the society. In this connection it may be advisable for the society to give consideration to enlisting the interest and support of in-

<sup>1</sup> Retiring President's address, Saint Louis, Missouri, March 29, 1946.



dustries dealing with plant products through issuing corporate memberships in our society. The experience of other scientific bodies has demonstrated the mutual benefits which can result from such an arrangement. The attainment of our majority should not mean termination of our growth. Each one of us represents a cell in the organism of the society; the development of this organism and the direction in which it is to grow in large measure depend upon the functioning of each individual cell.

Financially we are in a sound, though not affluent condition. Each year we have laid down a small annual ring to strengthen the total structure. The current inflation and low interest rates have been a source of much concern to the finance committee and to the business management. Every effort is being made to conserve our resources consistent with conservative administration, and especially to preserve the quality and usefulness of our journal. The devastation caused by the war places new and heavy responsibilities on the scientific institutions of this country. In this connection our society and our journal, in spite of their youth, must bear their share.

It has been the custom for untold ages on the occasion of a young man's coming of age, that his elders feel it their duty and privilege to give him sound and sober advice as to his conduct in manhood. I hardly feel equal to this rôle in the present instance. But it seems to me that this period of our life as a society offers opportunity for a little self-examination and study of what we are to make of ourselves and what we have to contribute to the world in which we are to live. More compelling for such an undertaking is the indisputable fact that in no epoch of human history has mankind been faced with such serious problems as confront us today. There is, moreover, the further fact that, directly and indirectly, many of these problems and conditions are the result of scientific discovery and endeavor. Mankind is looking to scientists to do their share in the huge task of solving the innumerable problems entailed in the establishment of a peaceful world. As a fundamental scientific discipline, plant physiology has some very real responsibilities in this connection. Our science had little to do with the destructive aspects of the war; it may have all the greater opportunity as a reconstructive influence for peace and good will.

It would be presumptuous in this gathering to try to define the objectives of plant physiology or to recount its accomplishments. As one scans the development of our science during the past century or so, with all due modesty, one is impressed with the large number of important fundamental discoveries which have emanated from plant physiological laboratories. Thus, osmosis, the composition of proteins, chromatographic analysis, to mention but a few, had their inception as a result of plant physiological investigation. And as one examines more carefully the foundations of our scientific structure one finds that there is hardly any branch of biological science which has not been fed with valuable raw material from plant physiology. In fact, being among friends, it may be said that plant physiology is at the center of plant biology. Time and again plant physiology has produced



buds which, when grafted to other disciplines, produced fruitful new branches on the tree of knowledge. In this manner important aspects of plant physiology have been grafted to horticulture, biochemistry, nutrition, medicine, and many other sciences. In the rapid development of modern science little attention is paid to origins; it is the fully matured fruit which commands attention on the market place, not the bud from which it sprang. Thus, while plant physiology can be justly proud of its contributions, these are all too frequently recognized only by the few comprising our select circle. It has been truly said that plant physiology is the "Cinderella of Botany."

Tempting as an analysis of this situation is, to penetrate a little more thoroughly into the underlying causes and influences, such a digression would take us far afield and beyond the memory of our young celebrant. As a phenomenon in the growth of a scientific discipline and for the purpose of clarifying the interrelationships of the various aspects of plant biology, a more thorough study of the history of plant physiology may be very rewarding. It would be quite appropriate for a committee of our society to begin its study of the place of plant physiology in biological research and in education with a consideration of its historical development in relation to other sciences.

Without presuming to have given this problem the study and thought it deserves, one aspect thereof is rather clearly revealed through a perusal of the contributions which have appeared in our journal since its founding and in other similar organs. During the past half century or so, at least since plant physiology became primarily an experimental science through the impacts of physics and chemistry, the researches in plant physiology have been primarily of an observational nature. That is, efforts were made to advance knowledge through direct, immediate discriminations of particular observations. This has been, of course, sound procedure. Experimentation with living things is vastly more complex and difficult than experimentation with inanimate systems. It was necessary to accumulate a great deal of experience in order to build a sound foundation for the structure of the science. There was so much to do, there were so many different phenomena and such a variety of organisms, that observation on the basis of experimentation was the order of the day and the on-coming generations of plant physiologists breathed almost exclusively the atmosphere of observation.

Without attempting to become too philosophical, it is nevertheless a matter of common experience that what we call science grows by the interaction of two orders of experience. These are observation and interpretation. The observations must be interpreted in terms of concepts. Neither can be said to be more valuable than the other for the creation of a real science. The one may modify the other, but what leads to further investigation and what we retain is usually in the form of concepts. It is the way of our conceiving of Nature.

Although there were some outstanding exceptions, it would appear that

plant physiology has been long on observation and a little deficient in the conceptual aspects. Through observation plant physiology has made a number of outstanding discoveries. It has all too often left the interpretative and conceptual development to other disciplines. Thereby it has lost the fruits of some of its most arduous labor and has hardly been given the recognition it deserves.

But we are not particularly concerned with the past; at twenty-one a youth sees only the future. Actually, plant physiology is in a very strategic position. Taxonomy, long primarily a descriptive science, gives indication of adopting experimental methods. Many aspects of ecology are applied physiology. Anatomy is giving increased attention to the function of the structures it describes with such minuteness. Genetics, long a highly developed experimental science, is impinging on physiology. The same is the case with phytopathology and with other branches of botanical science. Plant physiology has an opportunity as never before to play an important part in the development of fundamental concepts resulting from investigations which can be pursued most effectively through cooperative effort. And not only in relation to the botanical sciences can plant physiology be of value, but wherever plants or plant products appear our science has some contribution to make, as in nutrition, chemistry, and medicine.

Plant physiology has thus far been a rather highly specialized science. A specialty is of value only as a part of a larger field. Our situation in plant physiology is very much like that of the early days of railroading. A lot of railroads were built which started nowhere and ended nowhere in particular. It was only after these were built into great systems that we really began to go places. Plant physiology is a highly important link in the larger system of plant biology. We can help that system to grow, and as the larger system grows, we shall grow. As a nation we are inextricably involved in world affairs and in these world affairs food and many other plant products play a vital rôle. This, in part, at least, is the domain of plant physiology. We must contribute more not only to the scientific basis of agriculture and forestry, but also to that vast and rapidly expanding field of chemical industry which depends upon organic carbon. Plants are not only the basis of life; an ever increasing portion of our industry is drawing upon the plant kingdom for its raw materials. And while we are at it, let us not exclude coal and petroleum from our domain. Wherever there is need for carbon compounds the plant physiologist has an opportunity to make a contribution. This applies in the first place to that great array of substances which are used directly as they are derived from the plant such as drugs, oils, lacquers, resins, spices, perfumes, insecticides, beverages, *etc.* The knowledge of most of these materials came to us from primitive man. Science has hardly begun to explore the potentialities of the plant kingdom. Beyond this, however, lie the needs of the chemical industry for all kinds of raw materials in the form of carbon compounds. The much vaunted syntheses of organic chemistry are essentially the juggling

about of organic compounds into forms for which there is a demand. Organic chemists need the raw materials with which to carry out their juggling, and many of these raw materials have some plant as their ultimate source. In a large measure we are the custodians of this branch of knowledge. Within our speciality are the first opportunities to discover the things which other sciences, pure and applied, are seeking.

To the degree that we can make ourselves useful will our science thrive. We are living in the middle of the 20th century, faced with world cooperation and with world competition. In a large measure plant physiology lives still in the last century in respect to its efforts to integrate its results with those of other sciences and industry. In regard to demonstration of the usefulness of our science to society we have been outstripped by many other sciences, such as chemistry. In the case of the latter, the pure or fundamental aspects of this science have certainly not suffered because of contact with practical applications. On the contrary, the fundamental aspects of chemistry have if anything been furthered through these associations. And certainly so far as education is concerned, this has been quickened by virtue of the fact that so large a number of enterprising young people can look forward to a career in chemistry.

A characteristically youthful reply to much of what has just been said is: "How are we to get the support for such undertakings? Chemistry can do these things because of its great financial resources." To this there is but one answer. We shall have to do as the chemists did. We shall have to earn it.

It is not surprising that youth should be inordinately concerned with financial support of its scientific efforts. He is taught the ripened fruits of scientific work, rarely anything about the arduous labors which went into its culture. He sees fully grown industries, rarely their humble beginnings. His attention is constantly drawn to the full grown oak, rarely to the acorn. As a matter of fact, financial support is one of the last things that needs concern. Far more important is whether there is a discovery, a new observation, a novel relation between known facts, or a new product which merits support. Think of the astonishingly simple conditions surrounding the discovery of penicillin, and how rapidly the world was clamoring to give it support.

The basis for the support we have had thus far was in large measure earned by our predecessors who founded our science. We must re-earn our inheritance in order to possess it. But more than that, if our science is to grow we must add to this inheritance so that a greater estate can be passed on to our progeny. In order to accomplish this we must definitely adopt means which are in conformity with a changing world. In the new world order we cannot simply take it for granted that old methods will continue or suffice to meet our new responsibilities.

In the past we have lived primarily upon endowments and various forms of tax support. Youth takes for granted the living conditions under which

it has been raised, and usually regards them as the best imaginable, particularly if they have provided for him comfortably. There is no doubt that these methods of support have proved to possess many advantages. The urge has been to get more and more of the same kind of support. That is perfectly understandable and proper. We have, however, been inclined to overlook or to disregard the monastic and bureaucratic characteristics and influences which are inherent in this type of support. May we not with profit examine other methods of support for scientific research which have been evolved by some of our sister sciences and which have enabled these sciences to make such remarkable demonstrations of their usefulness to society? I refer to the general type of endeavor which has been developed by the Mellon Institute, and the Illinois Institute of Technology, and which in one form or another is being followed by many educational and scientific institutions, both here and abroad. The aim has been to integrate more directly scientific research and industry. The productiveness of these undertakings has been one of the outstanding organizational accomplishments in science. The public and the industry have both profited. Fundamental science has profited. Educational institutions and methods have been quickened and thousands of jobs have been created for ambitious young men and women. Thus far participating institutions have centered their efforts largely around the various branches of engineering, chemistry and physics. Certainly plant physiology has as valuable contributions to make to mankind. They are, no doubt, of a different nature, but they are quite as important.

It must be clear at the outset that in any arrangement involving industrial support high scientific and academic standards must be safeguarded. There is already available sufficient administrative experience to guide us through most of the shoals of such a course. All new ventures raise new problems. To scientists new problems are not a cause for despair, but rather a challenge.

Plant physiology deals with things most closely associated with the basic needs of man. Many of us could, I am sure, get along without a radio, without many electrical gadgets, and without cosmetics. But we could not get along without food, without houses and without the inspiration of a garden or of green fields and forests. Man is fundamentally a product of Nature. In the innumerable contacts of this man with the highly artificial environment he has created, plant physiology can be of inestimable value in helping him to lead a more healthful and rational life. In this connection there are many agencies and industries in need of plant physiological knowledge and skill. To the degree that we give and make ourselves useful to society will society support us.

Academically it may be argued, of course, that fundamentally it makes little difference how our support is obtained so long as the great domain of plant physiology is extended. It is definitely not my intention that co-operative support should in any sense replace the endowments and tax

support upon which we have relied. It is rather with the idea of broadening the base of our support, of enlarging the sphere of our influence that these suggestions have been made. As biologists, we are aware of the significance of the biocoenose for any particular organism. By the same token we cannot disregard our place in the mutual relations of a complex and changing society. The strength of a democratic society lies in the diversity of its social and intellectual institutions and in its ability to adjust itself to changing conditions.

The coming of age of any human or of any institution marks an important epoch. It means that the hazards of youth have been successfully overcome, and that there is sufficient virility and strength to look with confidence to winning out in the future struggle for existence. Our society has demonstrated its virility and its strength. It has lived through a desperate depression and a devastating war. It has the strength of youth to strike out in new directions and some background of experience to guide it. It has a sound inheritance, drawn from all parts of the world and from many branches of learning. Our society enters manhood over the threshold of a new era in the history of the world, equipped to carry its full share of responsibility, endowed with enterprise and strength, and, I am sure, with the whole-hearted support of its entire membership.

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# PHOTOCHEMICAL REDUCTION OF CHLOROPLAST GRANA

S. ARONOFF

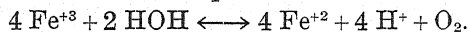
(WITH SEVEN FIGURES)

## Introduction

It has long been known that disintegration of green leaf tissue results in almost complete disappearance of photosynthesis. Indeed, any drastic treatment, of a type amenable to the isolation of various enzymatic systems, as intensive dehydration (above or below freezing), low temperature buffered extraction *et cetera* are of no avail. Attention has therefore been focused on some of the larger subcellular units known to function in the process, such as the chloroplasts.

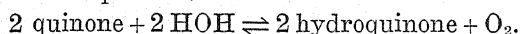
ENGLEMANN (6) showed, by means of bacteria motile only in the presence of oxygen, that oxygen is evolved only by the chloroplasts within cells. Later, BEYERINCK (3) used bacteria which can luminesce only in the presence of oxygen to demonstrate the evolution of oxygen from isolated chloroplasts. The maximal sensitivity of the latter method corresponds to the determination of oxygen having a partial pressure of  $10^{-8}$  mm. Hg. The sensitivity of the motile bacterial method is not known, except as the vague reference of PFEFFER (23), as a method capable of detection of one billionth of a mg. of oxygen. Using a method (quenching of a phosphorescent dye by oxygen) only slightly less sensitive than that of the luminous bacteria, but better adapted for routine quantitative measurements, FRANCK (11) has recently measured quantitatively the output of oxygen from isolated chloroplasts under various conditions.

Nevertheless, the activity of isolated chloroplasts, prepared by disintegration of leaf tissue, and differential centrifugation of the filtered mass, has been so low that until recently manometric methods could not be used to detect gas exchange. For contemporary routine biochemical investigations, therefore, their activity was nil. It was therefore of considerable interest that HILL (14) was able to show that on the addition of ferric oxalate to a solution of freshly prepared chloroplasts, considerable oxygen evolution occurred on illumination. Ferric ion was shown to be reduced to ferrous, and water was oxidized to oxygen. Quantitative measurements showed the reaction to follow the equation:



HOLT and FRENCH (20) showed that the reaction could be followed titrimetrically for the  $\text{H}^+$ .

A very much further advance was made when WARBURG and LÜTTGENS (24) demonstrated that an analogous reaction occurred using p-benzoquinone, not only with the chloroplasts, but with chloroplast grana. The reaction followed the equation:





The advance was of a two-fold nature: first, the complicated group of reagents required in the Hill reaction was replaced by a single substance; and second, the use of an organic oxidizing agent demonstrated the generality of the reaction. ARONOFF (1) further emphasized the generality of the reaction by the use of various types of quinones; *e.g.*, naphtho- and anthraquinones, to give the reaction with the grana, though at different rates, roughly proportional to their redox potentials in strong light.

BOICHENKO (4, 5) using a solution of 0.1 per cent. fructose in almost saturated basic Mg. acetate, has reported the evolution of oxygen from dried, as well as freshly isolated, chloroplasts. Unfortunately, we have been unable to confirm his results with manometric technique (the author used the leuco-dye method).

The work of FAN *et al.* (8) is also of interest since these workers have reported the reduction of benzaldehyde to toluol and the corresponding evolution of oxygen in light from green algae in the absence of external CO<sub>2</sub>.

The work to be described here forms the basis for the previously reported note. We have found that the reaction with quinone was given not only by the grana, but also by a clear solution containing chlorophyll in only minute amounts, and by lyophilized preparations of both. The subsequent sections therefore deal with (I) the method of preparation of the granules, solution, dried material, and experimental methods; (II) the rate of deterioration of the granules; (III) the rate of reaction of the various substances, as well as that of a leaf disc from which they were made; (IV) the action of poisons and narcotics; and (V) relation of the reaction to photosynthesis.

## Methods

### PREPARATION OF MATERIAL

GRANA.—In the following the use of the word "grana" is restricted to the product obtained under the conditions described, and is not intended to denote any morphological entity in normal tissue. This is a question that is, apparently, still open for further investigation. Market spinach was used as the source of granules. It had been hoped that tobacco chloroplasts, which can be prepared more abundantly (per weight of fresh tissue), and for the leaf of which more analytical data exist, could be plasmoptized and permit a comparison of chloroplast and granular reactions. Unfortunately, these chloroplasts do not disintegrate to any appreciable extent even in 48 hours of standing in distilled water, and spinach was found to be a ready source of "grana."

Spinach was purchased at a local market and was consistently of a better quality than that obtainable in a dozen or so other establishments in the immediate vicinity. It was equilibrated in a closed chamber to the temperature of the cold room (2–3° C.) within an hour, and all subsequent operations carried on therein, up to the moment of manometer manipulation.

Leaf lamina were separated from stems and large veins in amount sufficient to fill the container of a Waring blender without packing (ca. 100 gm. of turgid tissue); 175 ml. of phosphate buffer (pH = 6.5, 0.05 M) were poured into the blender (just enough to cover the blades). The blender was connected with a variable resistance and run 15 minutes at 55–57 volts in order to minimize the foaming. Below this voltage shearing action was insufficient. An additional 15 minutes were permitted for condensation of the foam to a dark green solution which was then filtered through two inches of cotton into centrifuge tubes. Although 15-minute centrifuging at 45 g. is sufficient to remove all chloroplasts, this material was run at 125 g. The resulting solution was relatively dark green, containing particles of a size at the limit of high-power microscopic resolution (ca.  $0.1\ \mu$ ) and having a chlorophyll concentration of ca. 0.1 mg./ml. The solution thus obtained was transferred into the celluloid tubes of a high-speed centrifuge and run at 6700 g. for 15 min. This is sufficient to precipitate almost all the grana and must be excessive, since the grana are rather tightly packed. The supernatant solution, yellow-green in color, was preserved for further work (see below). The grana themselves were washed twice while packed and then disintegrated with a stirring rod using a minimal amount of buffer. One hundred to one hundred forty ml. of initial granular material were thus concentrated to ca. 20 ml., the chlorophyll content of which normally ran ca. 0.75 mg./ml., often 1.0 mg./ml., and could be made as high as 2.5 mg./ml. The material thus obtained was used directly for experiments. The yield of material (in terms of chlorophyll) was 2–3 times as great for winter spinach (Dec., 1945) as spring (May, 1946), possibly because of the greater friability of the winter chloroplasts (whose yield, therefore, was negligible).

**SOLUTION.**—The yellow-green solution obtained from the high-speed centrifugation was clear, but showed a pronounced Tyndall effect. This material is referred to as "solution." It was dialyzed against distilled water for 36 hours, whereby additional chlorophyll-containing material was precipitated, and the yellow-green solution though very clear showed a very slight Tyndall effect. This material is referred to as "dialyzed solution." A hand spectroscope still showed the primary red absorption band of chlorophyll on looking through a 10-cm. path. The yellow color is due to the presence in high concentration of a yellow protein with maxima at 337 and 270  $m\mu$  (with a relative log K of 0.360 and 0.415 respectively). It is precipitable with ammonium sulphate and redissolves in saline solution. The yellow prosthetic group is so tightly bound to the protein, that no color is lost on dialysis. Although spectral indications would tend toward a flavone pigment, and a green coloration with ferric chloride shows the presence of hydroxyl groups, the characteristic orange color produced on reduction of a flavone or flavonone does not occur here. The high concentration is inferred from (a) the yellow color of a material absorbing in the far ultra-violet, and (b) the fifty-fold dilution required to obtain spectral measurement.

**DRIED.**—Both materials described have been dried in a lyophilizer made

by connecting in series a pump, trap in CO<sub>2</sub> solvent bath (in this case ethyl alcohol), and an Erlenmeyer having a male ground joint. The desiccation of 50 ml. of material, frozen before desiccation and kept frozen by the rapid evaporation of water, was effected overnight.

#### REAGENTS

1,4-benzoquinone was obtained from the Eastman Kodak Co. The material as obtained is sufficiently impure so that poor or negative results are obtained. A single sublimation is sufficient purification.

Potassium 1,4-naphthoquinone 2-sulphonate was prepared from  $\alpha$ -naphthoquinone according to the method of FIESER (9). It was twice recrystallized.

Potassium 1,2-naphthoquinone was prepared in similar fashion according to FIESER's method, and twice recrystallized according to his directions.

Potassium 9,10-anthraquinone 4-sulphonate was obtained through the courtesy of Dr. W. NUDENBERG of this department. It was found that the corresponding sodium salt, obtainable through the Eastman Kodak Co. is satisfactory as received.

Other compounds as poisons, narcotics, and additional substrates were used without recrystallization. Chlorophyll a + b was prepared according to the method of MACKINNEY (22) except for the drying of the product, which was performed in hi-vacuum, rather than by desiccator. It is believed that this is of value, since this material, after four years in a cork-stoppered tube at room temperature, still shows a good phase test.

#### EXPERIMENTAL METHOD

Gas exchange was measured manometrically in the usual fashion, using either Warburg or differential manometers, as the occasion required. Purified nitrogen (Ohio Chemical) was passed through alkaline anthraquinone-sulphonate to remove oxygen (ca. 0.1%), although this precaution was found to be unnecessary when using benzoquinone, and provided the standard atmosphere for the reaction. In filling the differential manometer it was found convenient to employ a three-way stopcock attached to the two manometer arms, so that equal pressure could be maintained in both arms. Following the 15-minute gas introduction, the inlet tube *above* the stopcock was removed, the vents on the manometer vessels closed, and the entire system permitted to attain equilibrium for 5 minutes. The manometer stopcocks were then closed and the three-way stopcock removed.

A 2000-watt tungsten lamp served as light source. The beam was spread by means of a cylindrical lens, collected, and reflected up through about four inches of water into the vessels. The light intensity in the center of the system at the level of the vessel bases was 19,000 lux. Inasmuch as it diminished somewhat on both sides, corrections were made in all runs on the basis of a linear relation between the light intensity and the rate of reaction (see section III). Diminution of intensity, when required, was accomplished by means of calibrated metal screens.

Total chlorophyll was measured by means of a Klett colorimeter, using Corning filter #2403. With an abscissa of  $\gamma$  of chlorophyll/ml. (chph./ml.) and an ordinate of density, the equation of the straight line was  $y = 8.52x$ . This equation is valid for  $x \leq 15$ . In actual practice, the suspension of grana was diluted one to ten with acetone, the mixture again diluted one to ten with acetone and then centrifuged 5 minutes at 100 g. to give a clear solution.

#### Rate of deterioration of granules

The data of both HILL and SCARISBRICK (17, 18) and KUMM and FRENCH (21) indicate a substantial rate of loss of activity of chloroplasts although the temperature of storage is not indicated in either case. The half-lives are of the order of 1.5 and 3 hours. Granules, stored at 2-3° C., were (assuming cold storage of chloroplasts) considerably more stable (see fig. 1),

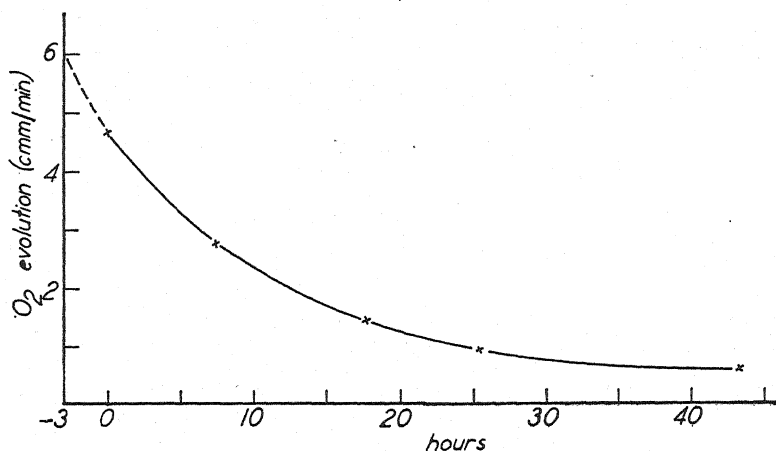


FIG. 1. The rate of deterioration of grana stored at 2° C. and illuminated 20 minutes at 19,000 lux.

the half-life (from the initial measurement) being eleven hours. As with chloroplasts, no external change in the appearance of the material was noted, though it could possibly have been noted in a change of the fluorescence yield. Because of the shape of the curve, the initial decrease is profound, there being an estimated 25-30 per cent. loss in activity of the isolated material during the time of preparation.

The loss of activity during an experiment was much greater and was presumably due to photolytic effects. Although experiments could be continued for 2-3 hours, the rate fell off markedly after 25-30 minutes.

It was noted, empirically, that the rate of deterioration was also a function of the quality of the material employed, as well as of the season. The figure shown is, therefore, of average material.

#### Rates of reactions of substances

The reaction had previously been shown to cease in boiled material, to decrease with time as material "deteriorated," and to diminish on narcotiza-

tion. Since each of the processes removes chlorophyll from the reaction, the first two by precipitation as colloidal, insoluble material, the latter by surface coating, it was desirable to show that the reaction did not occur with pure chlorophylls  $a + b$  in true solution. The reaction was carried out in both 95 per cent. ethyl alcohol and benzene saturated with water, with and without the addition of amino acids in both cases. No evolution of oxygen was noted. It is, of course, possible that aside from the twenty-fold reduction in amount of water present, and the replacement of hydration by alcoholation, that some essential component has been omitted.

#### LEAF DISC

The amount of photosynthesis of a leaf disc cut from material investigated was determined at various light intensities. For this purpose the leaf was floated in 0.1 M  $\text{KHCO}_3$  in an inlet atmosphere of 4 per cent. carbon

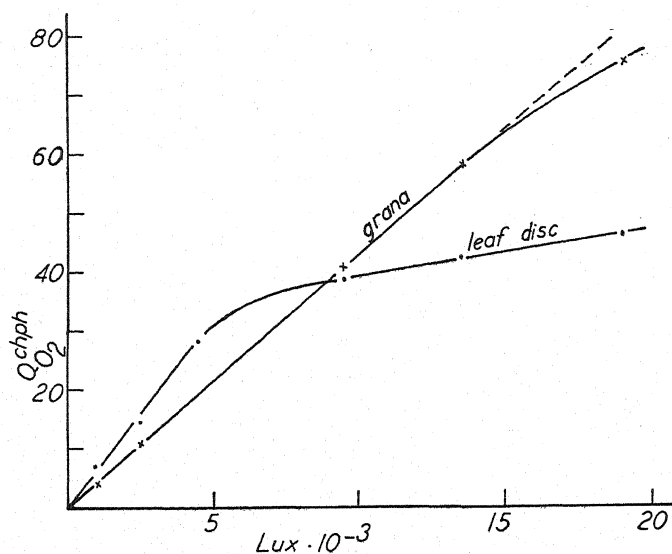


FIG. 2. Comparative rates of oxygen evolution of leaf disc with bicarbonate and grana with quinone.

dioxide, 96 per cent. nitrogen, and ca. 0.1 per cent. oxygen. The results are shown in figure 2. Among points to be noted are (a) the early light saturation, normally expected at ca. 17,000 lux for this material, and indicating some physiological disarrangement (of the time involved in the cutting, transit, marketing, and measurement, only the last is known); there is also a possible deficit of oxygen; (b) the low rate of evolution of oxygen; (c) the higher efficiency of the leaf at low light intensities than that of the grana (for the same amount of chlorophyll), especially in view of the very low redox potential of the oxidant in the leaf (carbon dioxide) and the high potential in the grana (p-benzoquinone). The quantum yields in our material would therefore be quite low. The fraction of the light

absorbed by the leaf per unit area is roughly equal to that of the grana suspension in the vessel.

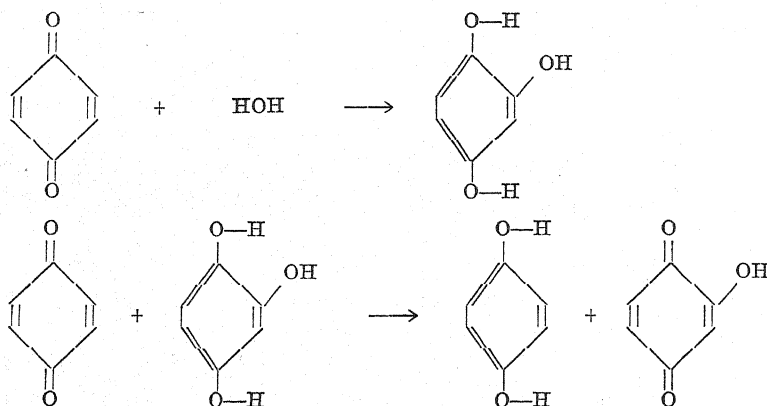
### GRANA

Measurements were performed, except in the cases noted, with *p*-benzoquinone. An excess, ca. 10 mg./5 ml. suspension was used. Using known quantities of quinone, the writer was not able to exceed 35 per cent. yield in a number of experiments, although WARBURG and LÜTTGEN (24) report 85 per cent. yields. The cause of the difference is not apparent. It is, for example, necessary that the quinone be purified. Alkalinity, light, *etc.*, cause a solution of purified (sublimed) quinone in Pyrex-redistilled water to turn orange-brown, with a color similar to that of quinhydrone. The deterioration of 1,4-naphthoquinone under similar conditions results in the formation of equal amounts of hydroxy 1,4-naphthoquinone + 1,4-naphthohydroquinone (9) which then combine in the usual fashion similar to quinhydrone formation, giving a colored substance.<sup>1</sup> Were this reaction to go to completion prior to the reaction with the chloroplast grana, it would, of course automatically remove 50 per cent. of the initial quinone. If it does not, the final yield with quinone nevertheless represents a competition between the two types of reactions for the quinone.

The rate of the reaction of the grana (fig. 2) with quinone is obviously a function of light intensity. Within the region of 13,000 lux it is linear for this size of granule. There is some indication it varies with the size. Diminution of activity as a function of time at higher light intensities may be correlated to some extent (as with normal photosynthetic systems) with photo-oxidation, but thermal inactivation resulting from the dissipation of the absorbed light energy and internal oxidation-reductions are undoubtedly more prominent factors since the partial pressure of oxygen is rather low.

Rates are plotted as cm. of oxygen evolved per mg. of chlorophyll per

<sup>1</sup> The reaction in the case of 1,4-benzoquinone would then be following FIESER (9):





hour =  $Q_{O_2}^{chph}$ , and varied between 45 and 100. Inasmuch as WARBURG and LÜTTGENS (24, 25) did not state their light intensities, beyond the statement that the reaction was completed within 30–60 minutes, it is impossible to compare results. The low quotient of the leaf disc itself may indicate that the starting material was in part to blame for the low rates. HOLT and FRENCH (20) have obtained values of 300–600 with their material using lower concentrations and higher light intensities. Obviously, had we used higher light intensities, we would have obtained higher yields (at least in the case of benzoquinone, though possibly not in the other quinones). Unfortunately, in the most recently available work from this group (11) it is impossible to compare any data, since the manner of denoting "activity" was not stated. A more rational "quotient" would thus also require the inclusion of the light factor.

It was of interest to determine the rate where chlorophyll concentrations were identical but particle size different. Chloroplasts were removed by centrifugation at 45 g. for 10 minutes. This has been found empirically sufficient to precipitate algae and pure tobacco chloroplasts quantitatively. By centrifuging an additional fifteen minutes at 45 g., a crop of "large" granules was prepared. The "smaller" granules were precipitated by centrifuging at 125 g. The colorimeter readings on the unextracted suspensions were 430 for the larger and 362 for the smaller, a difference due to the scattering factor. Neglecting the scattering factor (assuming equal light absorption) the rates of oxygen evolution were identical for solutions of equal chlorophyll content. Actually, however, because a larger fraction of the incident light was scattered by the smaller particles, the amount of absorbed light was less, and on the basis of the light absorbed (a quantal basis) their yield would be higher.

In contrast to the experience of HOLT and FRENCH (20) who used ferric oxalate, but in agreement with WARBURG and LÜTTGENS (24, 25) who used quinone, no carbon dioxide was obtained in the reaction.

Dried granules, resuspended in redistilled water, were found to be slightly more (58 per cent.) than one half as active as the undried material (using identical initial matter). Three days later the same material, kept at 2° C., was inactive. Dehydration is therefore insufficient for preservation.

#### SOLUTION

The solution is remarkable for its high activity, being about ten times as great as that of the grana; ( $Q_{O_2}^{chph} = 6-700$ ). Almost constant yields (at 19,000 lux) were observed for periods as long as two hours. Because of the low chlorophyll content it was possible to note a certain amount of destruction of chlorophyll in the course of the reaction, although the general character of the spectrum remained unchanged. The solutions responded to the naphthoquinones, but were erratic in regard to anthraquinone.

The solution, dried in a lyophilizer and redissolved in distilled water, retains its activity for weeks if kept cold while dried. This contrasts with

the marked diminution on storage of dried granules. Using dried solution, in one experiment with benzoquinone a constant rate of oxygen evolution was maintained after the fourth hour (fifteen minute illuminations each hour—total of one hour).

### Rates of reaction of substrates

#### RELATION OF REDOX POTENTIALS

It was reported in a previous note (1) that the rate of oxygen evolution was a function of the redox potential, using a series of homologous quinones. The figure therein is reproduced below (fig. 3). Because of the almost

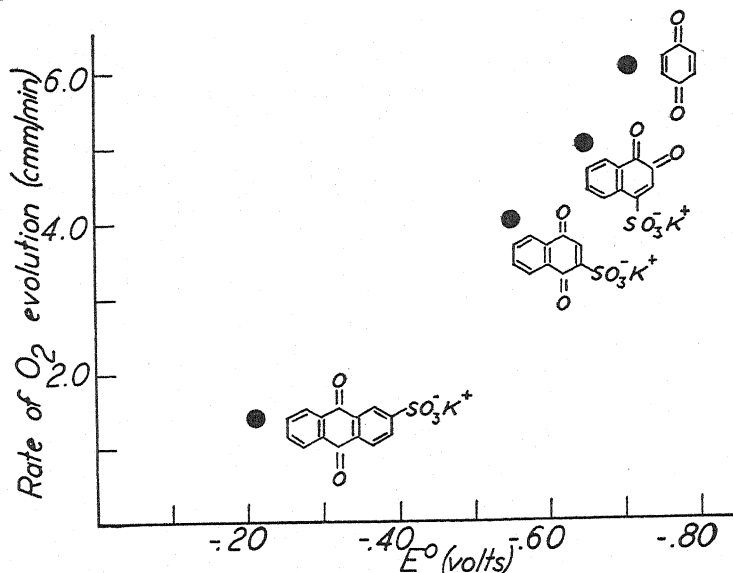


FIG. 3. Plot of rate of oxygen evolution by grana with various quinones (at 19,000 lux) against redox potentials of the quinones ( $E^\circ$ .)

linear relationship found between the oxygen evolution and the light intensity in the case of benzoquinone, it was presumed that this relationship held for the other quinones. Furthermore, the results were interpreted in the simplest manner as due to the difference in the rate of the reverse reaction (back reaction) for the different quinones. This explanation was in part based upon the known linear relationship between the redox potentials of leuco dyes and the rate of oxidation (2).

Since that time measurements have been carried out with the other quinones, determining the rate of oxygen production as a function of light intensity, the results being shown in figure 4. Measurements were performed as a differential effect; that is, using the same grana preparation, adding a known amount of benzoquinone to one vessel and an equimolar amount of naphthoquinone to the other. Furthermore, the two naphthoquinones were checked against each other in a similar manner; and, of

course, a curve was obtained for benzoquinone alone in the usual manner. Vessels with identical constants were used, and because of the small differences in evolution of  $O_2$ , a cathetometer was employed. The results indicate (a) that the order of the rates at low light intensities is the reverse of that at the high (with the possible exception of anthraquinone, where the very early inflection of the curve did not permit the determination of reliable data) and (b) that the curves for the naphthoquinones were linear at only the lower light intensities.

The change in character of the slopes from a straight line to that of a curve appears to indicate that at least one dark reaction occurs in addition to that of the light reaction.

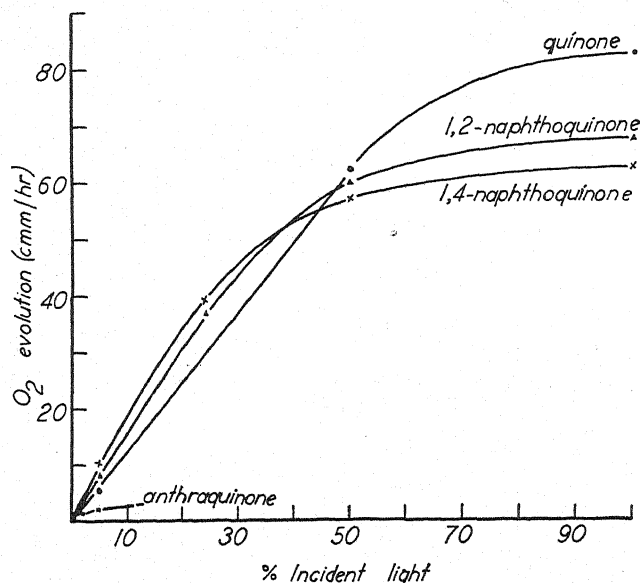


FIG. 4. The rate of oxygen evolution by grana with various quinones as a function of light intensity.

No explanation will be attempted at the present concerning the order of the rates at low light intensity, since this involves further experimentation (as a function of time, *etc.*)

At the higher light intensities there is a choice of two possible explanations. The first, which would be valid if true saturation occurred, would be that of the limitation of the rate by an enzyme with a finite turnover number, worked with insufficient rapidity to keep up with the light reaction. This enzyme would in this instance have different turnover rates for the different substrates and the relation to the potentials indicated would, in a sense, be fortuitous, although in the long run possibly ascribable to the same structural properties which determine potential difference in compounds. The second possible explanation for a diminution in the rate is the increased rate of the reversal of the light reaction with increasing concentration of the

products of the forward reaction. In this instance, saturation, though approached, would never be reached, on the basis of a bimolecular reaction. Unfortunately, the instability of the material at high light intensities does not lend itself to a ready choice between the mechanisms.

#### NON-QUINONES

Various other substances have been tried as oxidants; *e.g.*, using salicylaldehyde, a small amount of oxygen is evolved initially, ceasing within a short time, and bleaching of chlorophyll is considerable. Benzaldehyde, although much more susceptible to oxidation, evolves a greater amount of oxygen prior to cessation, than does salicylaldehyde and the chlorophyll bleaching, though considerable, is less severe. Salicylic acid was most effective as a bleaching agent, and no evolution of oxygen was detected. A number of other substances gave very little response: fructose, dehydroascorbic acid, methyl alcohol.

Attempts to obtain a wider indication of the possible effect of potential using a series of dyes (2,6-dichlorophenol indophenol) were abandoned because of the large fraction of light absorbed by the amount of dye required to produce a nominal manometric effect.

The grana decompose urea peroxide in the dark, as does catalase. This probably denotes a high dissociation of the substrate into urea and hydrogen peroxide. Butadiene monoxide (3,4-epoxide, 1-butene) also gave no oxygen.

It is interesting to note that benzoyl peroxide evolved oxygen to some extent, with no observable liberation of gas in the dark.

#### ALGAE

FAN *et al.* (8) have reported that green algae are able to evolve  $O_2$  in the light and explained their results as the reduction of benzaldehyde to the corresponding alcohol. Their experiments were complicated by (a) the relatively large amount of metabolic carbon dioxide present in the dense suspensions, permitting a release of oxygen due to normal photosynthesis as great as that due to the reduction of benzaldehyde; (b) the apparently usual presence of air during the experiment, resulting in the oxidation of the aldehyde; and (c) the slow rate of entry of the aldehyde into the algal cells.

We have attempted to repeat our experiments on the alga, *Scenedesmus*. As with the grana, the algae were in an atmosphere of nitrogen (+ 0.1%  $O_2$ ), and bathed in a phosphate buffer identical with that used for grana. The sole carbon dioxide available was that produced by anaerobic metabolism. The density of the suspension was far from that resulting in total light absorption. Since an excess of quinone above that of a saturating solution did not increase the rate of oxygen evolution, it was presumed that the rate of quinone entry into the cells limited the oxygen evolution. Under these conditions, oxygen was evolved only in the light. The same algae, under the same conditions, without quinone did not evolve oxygen.

## Poisons and narcotics

One of the anomalies of the Hill reaction is the inaction of the usual poisons. Thus, cyanide is of no effect, nor is azide. The former is believed to affect the carboxylating enzyme in normal photosynthesis, and since there is evidence (14) to indicate that this enzyme may be located outside the chloroplast, the inaction of cyanide may thus be understood. The same cannot be said of azide<sup>2</sup> which would presumably act on the oxygen-liberating enzyme. Even more pronounced is the lack of activity of hydroxylamine.<sup>1</sup> We had, indeed, hoped for some activity, since, from the point of view of potentials, hydroxylamine should be a good oxidizing agent. Actu-

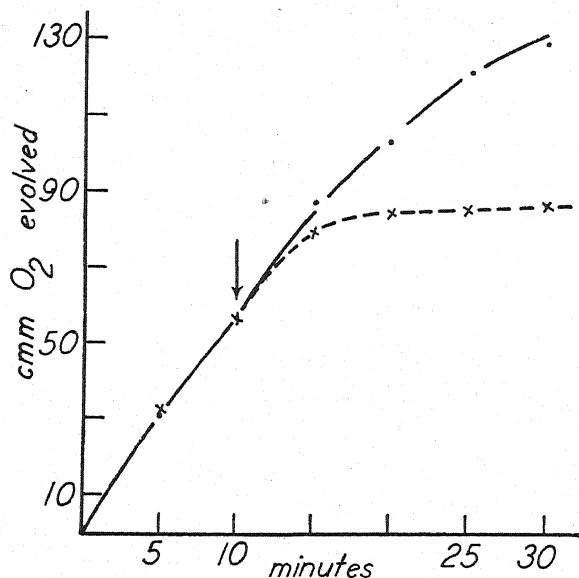


FIG. 5. Inhibition of the evolution of oxygen by grana and quinone by o-phenanthroline. — control; - - - - o-phenanthroline.

ally, it is a very poor one and is, in fact, used as a reducing agent in normal practice. We have found no evolution of oxygen with this substance, except for a slight initial activity which soon ceased. This activity produced may be due to reaction of the amine with naturally occurring ketones or aldehydes. Thus, the primary action of hydroxylamine in the presence of quinone is to form the dioxime. This is accompanied, however, by considerable evolution of gas, apparently nitrogen (and all, of course, without relation to light or enzymes).

The action of o-phenanthroline was found, in agreement with WARBURG and LÜTTGENS, to be an effective narcotic (fig. 5). The almost complete sup-

<sup>2</sup> FRENCH, *et al.* (12) have noted the action of hydroxylamine and azide as that of poisons. This is at variance with the results of both HILL and SCARISBRICK (16) on chloroplasts, and with ours on granules. They also mention the poisonous effect of fluoride, which HILL and SCARISBRICK found was not a poison. Furthermore they class Duponol (presumably sodium lauryl sulphate) as a poison. Undoubtedly this detergent acts as a surface agent and should therefore be classed as a narcotic.

pression of photoreduction makes it a very powerful inhibitor (although this may be due to its relatively large concentration compared to other narcotics). The substance is termed a narcotic despite its ability to be a poison by virtue of its chelation with ferrous ion, since WARBURG and LÜTTGENS (24, 25) found that its inhibiting action can be removed merely by washing and resuspending the grana.

The most common photosynthetic narcotic, phenylurethane, was found, in agreement with HILL's data on chloroplasts to be effective with grana (fig. 6). One notes, however, that with chloroplasts, by using sufficiently high concentrations, almost complete suppression of activity can be obtained with both ethyl- and phenylurethane. In the case of grana, using a saturated solution of phenylurethane, only two-thirds inhibition results. An

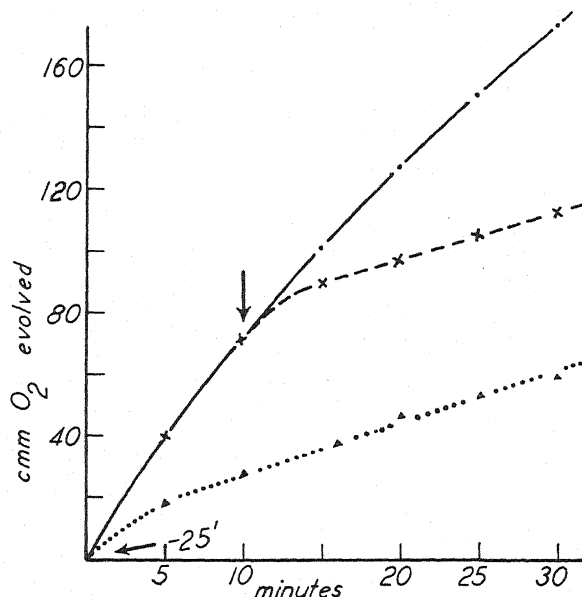


FIG. 6. Inhibition of the evolution of oxygen by grana and quinone by phenylurethane. — control; - - - phenylurethane ( $2.0 \times 10^{-3}$  M); . . . . phenylurethane (excess; crystals).

additional difference, one which occurs with grana and apparently not with chloroplasts, is the constancy of the inhibited rate. The time effect with plastids is considerable, indicating a considerable diffusion factor through the membrane to the effective loci. The lack of an increased effect of excess phenylurethane (including a previous twenty-five minute incubation) indicates that the two-thirds rate diminution is the limit with this substance.

The principle of narcotic action is well illustrated by comparison of thymol with phenylurethane. Using identical concentrations ( $1.2 \times 10^{-2}$  M) in which phenylurethane diminished the control rate by 55 per cent., thymol diminished the rate by 80 per cent. (fig. 7). The distribution coefficient between olive oil and water for phenylurethane is 150, and for thymol, 600



(19, p. 357). On the assumption that the effectiveness of a sub-limiting concentration of inhibitor is then determined by its solubility in the (oily) matrix of the grana, one would have inferred that thymol should be about four times as effective as phenylurethane.

It is to be noted, though possibly at present it is considered coincidental, that the limit of effective narcotic action is attained when the ratio of phenylurethane is one. This implies that with less concentrated grana solutions, less concentrated phenylurethane will be required to produce a given effect. This experiment has not been performed.

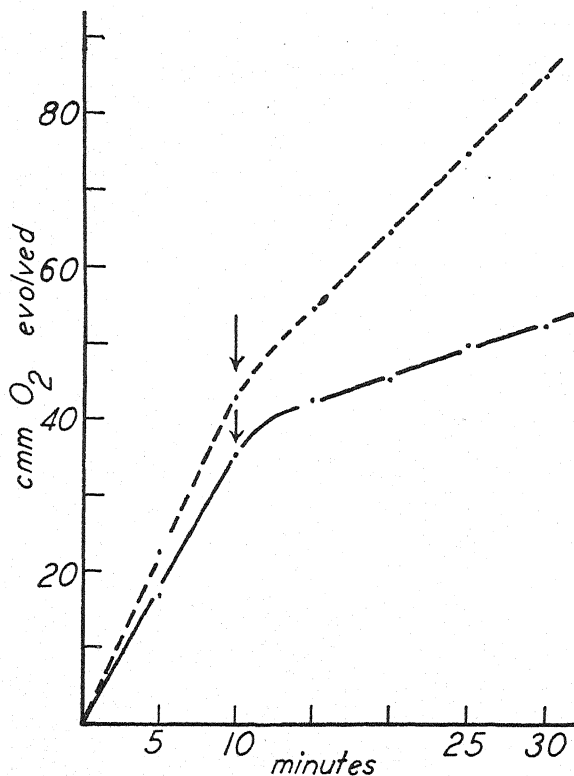
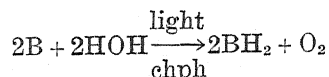


FIG. 7. Inhibition of the evolution of oxygen by grana by submaximal amounts of phenylurethane and by thymol; - - - - phenylurethane ( $1.2 \times 10^{-2}$  M); ——— thymol ( $1.2 \times 10^{-2}$  M).

#### Relation of the reaction to photosynthesis

HILL (14) correlated the activity of iron in photoreduction in his isolated chloroplasts with a specific material reacting as a catalase-like enzyme. That various types of quinones (1, 24, 25), aldehydes, *etc.*, react in a similar manner with products of the chloroplasts, the grana, indicates a generality of the reaction greater than previously thought. It is, of course, possible to write a general equation describing all of the above reactions in a manner

identical to the generalized equation for photosynthesis suggested by van Niel, *viz.*



In particular the oxidant B is represented by relatively strong oxidizing agents, as ferric ion, or quinone, whereas in normal photosynthesis, the oxidizing agent is the very weak carbon dioxide. In both cases the oxidant is water, although this has not been shown for photoreductions where a reducing agent, in addition to that of water, is required.

It is not possible to say at present, however, that this reaction is the "last half of photosynthesis." Certainly it is not the "first half" since this occurs in large measure outside the chloroplast. The lack of effect of the poisons (with the possible exception of o-phenanthroline) indicates that the normal enzymatic system responsible for the release of oxygen is not present, or at least is inoperative under the conditions used in the present work. Indeed, as indicated previously, we cannot even be certain that the enzyme(s) involved in the stabilization of the primary photolytic products is operative. The only mechanism of which we may be certain is the fission of water in the light reaction; and it is this step, the "middle" of photosynthesis, which the reactions may have in common.

The nature of the transfer of light to chemical energy is, however, of the utmost importance and significance, and therefore worth continued study.

### Summary

1. Methods of preparation of grana and of a clear solution which can be used for the evolution of oxygen by photochemical reduction of quinones have been described.

2. The rate of deterioration of the grana, stored at 2° C. is such that a half-life of about eleven hours is indicated. Approximately 25-30 per cent. of the activity is lost in time of preparation.

3. The rate of oxygen evolution with grana and using quinone is only a tenth that of the solution (at maximal light intensity) based on the amount of chlorophyll. The solution is much more stable in light than grana. Both lyophilized grana and solution are active on rehydration. The lyophilized solution is more stable than the grana.

4. The rate of oxygen evolution varies with different quinones (benzoquinone, 1,2-naphthoquinone, 4-sulphonate, 1,4-naphthoquinone 2-sulphonate,  $\beta$ -anthraquinone sulphonate). At lower light intensities the order of rates is the reverse (with the possible exception of anthraquinone) of that at the high light intensities. Saturation, or an approach to it is indicated at high light intensities. Various other substances give small effects: benzaldehyde, benzoyl peroxide, and salicylaldehyde. Others give very little effect: fructose, and dehydroascorbic acid. Others which might have been

expected to be active give none: butadiene monoxide and salicylic acid. Urea peroxide is decomposed in the dark by both the grana and a solution of pure catalase, indicating dissociation of the urea peroxide to urea and hydrogen peroxide.

5. The photochemical reduction of quinone is inhibited strongly by o-phenanthroline, by phenylurethane, and by thymol. Complete inhibition by phenylurethane does not occur with saturated solution; the degree of inhibition is not increased by incubation or with excess material. Thymol is more fat-soluble than phenylurethane and is more effective as an inhibitor at the same molar concentration.

6. A brief discussion of the relation of photochemical reduction with various substances by grana to photosynthesis is given.

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# AN APPARATUS AND METHOD FOR THE MEASUREMENT OF THE RESPIRATION OF COTTONSEED

A. M. ALTSCHUL, M. L. KARON, AND P. J. FENN

(WITH TWO FIGURES)

## Introduction

In the course of an investigation to determine the relationship of the respiration of cottonseed to the other processes which take place during storage, studies were made of the effect of moisture content, maturity, and inhibitors on respiration, rate of free fatty acid formation, and pigment changes in the oil. For these studies, a series of measurements was required on about fifty different samples of cottonseed over a period of one year.

In view of the large number of analyses needed, an apparatus designed to obtain accuracy and speed was built to follow the changes of the oxygen and carbon dioxide concentrations in the atmosphere surrounding the seeds. This apparatus and the methods used to make and record the measurements are described below.

## Methods

A sample of seeds for which a measurement of the respiration rate is desired is stored in a stoppered flask. The size of the sample and the length of the storage interval are determined by the estimated respiration rate of the seed sample. Since an accumulation of carbon dioxide in the atmosphere surrounding the seeds inhibits respiration (2), a period of storage is selected that will not permit the development of more than a 3-per cent. concentration of carbon dioxide in the flask at the time of analysis.

At the conclusion of the storage period, a sample of the atmosphere in the flask is withdrawn and analyzed for both oxygen and carbon dioxide content. This is accomplished by the successive removal of carbon dioxide and oxygen accompanied by measurements of the resulting changes in pressure at a constant volume. The gas sample is first dried, then drawn into a measuring chamber of fixed volume kept at constant temperature, and its pressure measured. The percentage of carbon dioxide is then determined by passing the gas sample several times through a tube of Ascarite,<sup>1</sup> after which it is pumped back into the measuring chamber. The percentage drop in pressure represents the carbon dioxide content of the sample. Oxygen is determined on the same sample by passing the carbon dioxide-free gas several times over a heated copper screen before pumping it back into the measuring chamber. The difference between the percentage of oxygen in a sample of air and in the sample which was analyzed is equal to the percentage of oxygen consumed by the respiring seed. After each oxygen analysis, the copper is regenerated by passing hydrogen gas over the heated oxidized copper screen.

<sup>1</sup> A commercial preparation of asbestos coated with sodium hydroxide.

## Apparatus

Figure 1 is a schematic drawing to scale of the apparatus used for the gas analysis, and figure 2 is a photograph of the apparatus. All of the

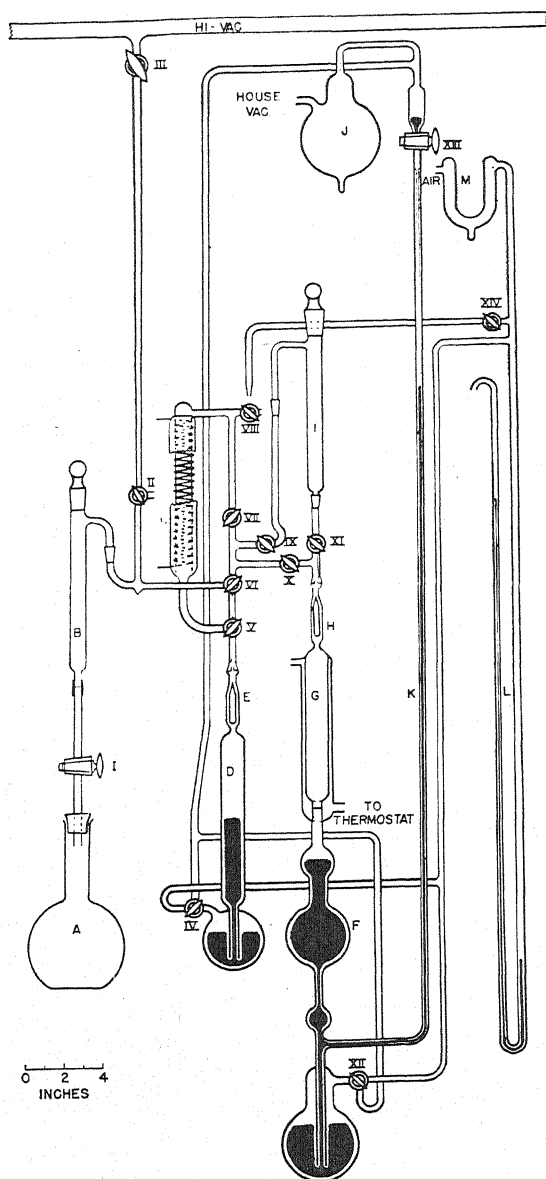


FIG. 1. Diagram of respirometer.

connections in this apparatus were made with standard tapered ground glass joints. Vacuum-tight seals were obtained by cementing the ground glass joints with a phthallic anhydride resin. It will be noted that this



apparatus is similar in many respects to the one described by BAMFORD and BALDWIN (1). It was found desirable, however, to sacrifice some of the

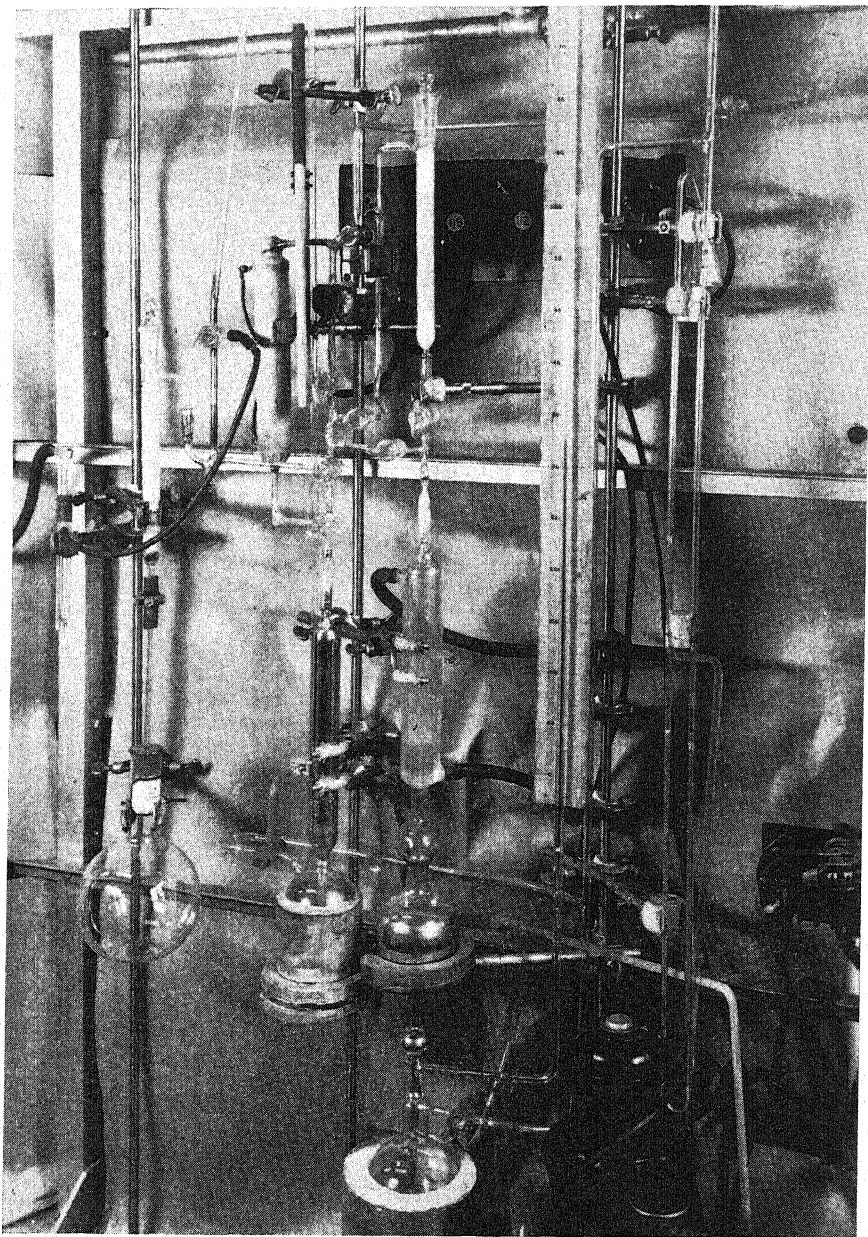


FIG. 2. Respirometer assembly.

accuracy obtained by BAMFORD and BALDWIN in favor of simplification of operations to obtain the speed necessitated by the number of analyses to be

conducted. Nevertheless, almost negligible difference existed between the oxygen content of air found with the apparatus used in this investigation ( $20.93 \pm 0.05\%$ ) and that found by BAMFORD and BALDWIN ( $20.94 \pm 0.04\%$ ).

### Method of analysis

Description of the procedure followed with a typical gas specimen will serve to illustrate the method of analysis. The symbols used refer to those indicated in figure 1.

#### MEASUREMENT OF THE INITIAL PRESSURE

The respiration flask, A, is connected by paraffin-covered rubber tubing to the tube B which contains phosphoric anhydride layered on glass wool. This tube serves to remove water and volatile bases. With stopcock I closed, the entire apparatus is evacuated to approximately 0.05 mm. pressure by means of a "Megavac" pump. The gas sample is admitted slowly into the measuring chamber, FG, through B and the system of stopcocks I, VI, X. Stopcocks X and XI are closed; mercury is forced up through bulb, F, to the mark in the capillary tubing; and the pressure on the manometer, K, is read to the nearest half millimeter. The quantity of gas admitted is such that the initial pressure is slightly less than atmospheric pressure. The chamber, G, is maintained at  $26^{\circ}$  C. by circulating water from a constant temperature water bath through the surrounding jacket; and the vacuum, in the manometer, K, is renewed daily by forcing the mercury up through the stopcock XIII, before closing it.

#### MEASUREMENT OF PERCENTAGE OF CARBON DIOXIDE

After the initial pressure has been determined, the sample of gas is passed through the tube containing Ascarite, I, into the Toepler pump, D, through the system of stopcocks IX, VI, and V. Both ends of the tube I contain small amounts of magnesium perchlorate to absorb any moisture produced during the removal of carbon dioxide by Ascarite. Compressed air and vacuum (5-mm. laboratory vacuum line) are used to regulate the level of mercury in the chamber, G, and in the Toepler pump. The air, as obtained from the laboratory source, was under 80 pounds' pressure. This pressure was reduced by the use of the bleeding stopcock XIV, to that required as measured by the manometer, L. The float valve, H, prevents mercury from rising into the capillary lines. A similar valve, E, above the Toepler pump affords protection to the capillary lines above the pump.

When all of the gas has been forced out of chamber G into the Toepler pump, the flow is reversed and the gas is pushed back again through the Ascarite absorption tube. The process is repeated twice more, so that, in all, the sample of gas is passed over the Ascarite six times. At the end of the sixth operation, all of the gas will have been forced out of the Toepler pump, and the level of the mercury in the measuring chamber is set at a point below bulb F. The gas remaining in the absorption tube, I, and in

the capillary lines is pumped back into the system FG by the use of the Toepler pump. Four pumpings are sufficient to remove all the residual gas from the lines. With stopcocks X and XI closed, the level of the mercury in the measuring chamber is again brought to the mark in the capillary tube below G and the pressure is measured. The fraction of carbon dioxide in the gas sample is equal to the drop in pressure divided by the initial pressure.

#### MEASUREMENT OF PERCENTAGE OF OXYGEN

Oxygen is removed from the carbon dioxide-free gas by passing the gas over the heated copper screen in tube C into the Toepler pump through the system of stopcocks X, VII, and V. Tube C is electrically heated by means of 30 feet of size 22 Chromel-A wire (0.999 ohm per foot) wound around the outside of the tube. Fifty volts A.C., taken from a variable transformer, are sufficient to give adequate heat for both the removal of oxygen during the analysis and for the subsequent reduction of the copper oxide by hydrogen. The operations used for the removal of oxygen in the sample and for the return of all the residual gas to the measuring chamber are similar to those carried out for the determination of carbon dioxide. When the analysis of the sample has been completed, the copper surface on the screen is renewed by passing hydrogen gas into the heated tube, C, through the system of stopcocks II, VI, V, and VIII.

The drop in pressure resulting from the removal of oxygen in the sample divided by the initial pressure is equal to the fraction of oxygen in the sample. The change in oxygen pressure due to the consumption of oxygen by the respiring seeds is obtained by subtracting the percentage of oxygen obtained by analysis from the oxygen content of the air (20.93%).

#### Typical results

A set of typical recordings of experimental data and calculations of the results of the gas analysis are presented in table I. In the case of analysis No. 1, the oxygen was first removed, followed by carbon dioxide; in the second analysis the order of removal of the gases was reversed. It will be noted that the order of removal of the gases has no significant effect upon the accuracy of the results.

TABLE I

MEASUREMENTS AND CALCULATIONS OF THE RESULTS OF A TYPICAL GAS ANALYSIS

ANALYSIS NUMBER	PRESSURE			CARBON DIOXIDE EVOLVED	OXYGEN CONTENT	OXYGEN CONSUMED
	INITIAL	AFTER OXYGEN REMOVAL	AFTER CARBON DIOXIDE REMOVAL			
1	mm. Hg. 766	mm. Hg. 610.5	mm. Hg. 605.5	% 0.65	% 20.30	% 0.63
2	739	584.0	734.5	0.61	20.36	0.57

Starting with data of this type, it is possible to place the results of all respiration experiments on a comparable basis by reducing the analytical values for carbon dioxide and oxygen to the number of cubic centimeters of gas at standard temperature and pressure that is evolved or consumed per day by one gram of seed (moisture-free basis). To effect this transformation, it was necessary to measure the volume of the free air space in the respiration flasks, so chosen that the volume of free space was constant for each size of seed sample. For a 10-gram sample of seed the air space was 1108 cc.; for a 25-gram sample, 1079 cc.; and for a 50-gram sample, 1032 cc. Corrected to standard conditions, the volume of gas in the above flasks was 1022 cc., 995 cc., and 953 cc. for 10-, 25-, and 50-gram samples, respectively.

The complete calculation of the respiration rate of the sample illustrated in table I is shown in table II.

TABLE II  
CALCULATION OF RESPIRATION RATE

RESPIRATION INTER- VAL	WEIGHT OF SAMPLE	MOIS- TURE CON- TENT	WEIGHT OF DRY SEED	CARBON DIOXIDE EVOLVED	OXYGEN CON- SUMED	CARBON DIOXIDE EVOLVED	OXYGEN CON- SUMED	CARBON DIOXIDE EVOLVED PER GRAM PER DAY	OXYGEN CON- SUMED PER GRAM PER DAY
<i>days</i>	<i>gm.</i>	<i>%</i>	<i>gm.</i>	<i>%</i>	<i>%</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
39	50	10.1	45.0	0.63	0.60	6.0	5.7	0.0034	0.0032

### Summary

1. An apparatus, developed for the determination of the respiration rate of cottonseed, is described. Carbon dioxide and oxygen are successively removed from the gas sample, and the changes in pressure of a fixed volume of the sample, occasioned by removal of the gases, are used to calculate the percentage of carbon dioxide and oxygen in the sample.

2. The results of the respiration rate measurements are expressed in terms of the number of cubic centimeters of carbon dioxide or oxygen, at standard temperature and pressure, evolved or consumed per gram (moisture-free basis) of seed per day.

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## THE RESPIRATORY PROCESSES IN PLANT CELLS IN RELATION TO THE FORMATION OF COACERVATES

J. DUFRENOY AND H. S. REED

(WITH TWO FIGURES)

Life depends on the existence of a water phase within the cytoplasm. Generally the water phase, as represented by the vacuolar solution is optically empty: in whatever condition the solutes are, they are hydrated and randomly distributed so that they appear to be in solution. The vacuolar solution, however, may itself become the site of a separation of phases through coacervation, as we intend to show by the data to be presented.

Coacervation may occur in the vacuolar solution of plant cells under the influence of various agencies apt to unbalance cell respiration, such as zinc deficiency, virus or fungal infection. Auto complex coacervates, made up of a central core of polymerized phenolic compounds, surrounded by phosphatides, once formed, appear as permanent structures in the cell and have frequently been misinterpreted by observers. Recent advances in cytochemistry emphasize that the vacuole is the reaction chamber of the cell. The process of coacervation in it affords an unusual opportunity to visualize one of the delicately balanced equilibria so essential for life (13).

The polyphenols, chiefly catechol, which are so widely and almost universally distributed in the vacuolar solution of plant cells, have during these recent years been recognized as the most important mediators between the metabolites furnishing the hydrogen and the atmospheric oxygen accepting it in the process of aerobic respiration. In the normal "compensated" respiration these compounds are reduced back to the original condition of polyphenols as fast as they are dehydrogenated to quinones. Therefore they retain their original physical as well as chemical properties.

When the respiration is "non-compensated" the phenolic compounds, having been dehydrogenated to quinones, no more have made available to them the activated hydrogen that can reduce them to their original condition (9). Instead of being reversibly reduced, they become subject to further oxidations resulting in concomitant changes of their physical conditions. Some of the linkages which should normally have been C-OH linkages become C-C linkages, meaning linkages between the C of different molecules, building large molecular weight units, or polymers, which assume the consistency of a gum, and the color of a pigment—either the brownish-red phlobaphenes, or the vivid red anthocyanols.

The phenomenon of molecular aggregation, or polymerization, was correlated with changes in chemical affinity by BERTHELOT (2), GULDBERG and WAAGE (8) and PASTEUR (11). BERTRAND (3) applied his newly acquired knowledge of oxidases to explain the concomitant oxidation and polymerization of Boletol and other phenolic derivatives. COMBES (4) recognized that



when anthocyanic pigments are formed, oxygen is fixed by the organs which become red; then those organs are the site of enhanced oxidative phenomena. Conversely, when anthocyanic pigments disappear, the organs whence they disappear release oxygen. It is possible to demonstrate an aggregation of oxidizable molecules into more or less soluble gummy masses in cells which are the sites of unbalanced or non-compensated respiration. The phenomenon may be correlated with a process of dehydrogenation not only *in vivo* but *in vitro*.

#### Relation between coacervates and the non-compensated respiration

Diseased tissues which evidence discoloration, absorb more  $O_2$  than normal, and release less  $CO_2$ ; oxygen acts as a hydrogen acceptor whereby the phenolic compounds become dehydrogenated into pigments. Oxygen, however, is not fixed by the pigment. As phenolic compounds, such as catechol, normally dispersed in solution in the vacuolar sap, are being dehydrogenated, due to non-compensated respiration, both the rH and the pH of the vacuolar solution would shift; correlatively the behavior of the compounds toward water will be altered. Molecules of polyphenols, originally dispersed at random in the vacuolar solution, become grouped into larger units of polymers and those units may not remain distributed at random, but may become more densely congregated at certain loci.

This unequal distribution of phenolic compounds within the vacuolar sap may be achieved through one of two ways:

1. The vacuolar sap in the cell becomes apportioned to different vacuoles, some of which may contain phenolic compounds, others not. In other cases the initially single vacuole containing the whole of the vacuolar solution in the cell may bud out, at its periphery, a number of drops which will become individualized into many small vacuoles. These drops may contain a solution richer in phenolic compounds than the original solution, in which case the central vacuole may be described as secreting or excreting phenolic compounds, or, on the contrary the exudate may be devoid of phenolic compounds, in which case the original vacuole excretes water, and concentrates the phenolic solution.

2. Instead of being achieved through the fragmentation of the vacuole, the concentration of the phenolic compounds can occur in the vacuole itself which retains its full volume, while the phenolic compounds contract spontaneously and become condensed as a mass floating in the vacuolar liquid, now impoverished in colloids.

Many investigators actually stained coacervates with dyes like neutral red, but failed to realize that they were floating in the vacuolar solution. MAST (10), studying the relations between the kinds of food, growth, and structure, reported that *Amoeba proteus*, which had fed for several days exclusively on Chilomonads, contained an extraordinarily large number of spherical bodies which showed a definitely differentiated structure;



namely, a central mass surrounded by a distinct, though fragile, shell which was covered with a thin layer of oily substance. In solutions containing neutral red the outer layer became crimson in color, but the central portion and the shell were not stained by the dye. Coacervates thus express the ultimate effect of a gradient in the distribution of the vacuolar materials. The phenolic compounds as they lose hydrogen develop ability for C linkages; hydrogen ties up into  $H_2O$  or  $H_2O_2$ ; less and less hydrogen is available to redisperse the polymerized phenolic compounds, or rather the dehydrogenase systems (whereby hydrogen should have been made available) fail to perform, either because the active phosphorus linkages have been broken or because the active SH groups have been oxidized to disulfides.

The loosening of phosphorus linkages concomitant with the dispersion of the dehydrogenase systems, provides for a supply of ionic  $PO_4$  which goes into the vacuolar solution, there to become incorporated into the amphoteric lipid complexes at the boundary of the auto complex coacervates.

The non-compensation of respiration, following dispersion of dehydrogenases, lack of active hydrogen for rehydrogenation of coacervated phenols, immigration of  $PO_4$  ions into the vacuoles onto the boundary of the coacervates, can be experimentally induced by treatment with agents such as salicylaldehyde interfering with the normal activity of dehydrogenases.

#### Coacervates in plant cells

The effect of disrupting the dehydrogenase system was convincingly shown in the experiment where sugar cane was treated with 100 p.p.m. sodium xanthogenate. Within a few hours living cells were in a condition represented by figure 1. Following treatment with the molybdenum reagent, the nuclear mass gave a diffuse reaction for phosphorus, mitochondria were swollen, phenolic material was coacervated within the network of the phospholipid and was floating in the vacuolar sap. This condition can be interpreted as the result of the disequilibrium between the dehydrogenase system and the oxidase system. The former being more sensitive, most reagents are likely to induce a non-compensated respiration as expressed by coacervation. This will explain the common occurrence of coacervates in pathological tissues as will be shown later.

About 1940, in some of the sugar cane fields of Louisiana were first detected a few stalks affected by an infectious disease, which was named "chlorotic streak." ABBOTT and INGRAM (1) obtained evidence that it is a virus disease and showed that it could be transmitted by a leaf-hopper. A red discoloration of vascular bundles at the nodes was revealed when the affected stalks were split lengthwise. Freehand longitudinal sections of the nodes showed in most perivascular cells a large coacervate, the contents of which gave positive reactions for phenolic compounds, and most specifically gave the positive Scudi reaction for pyridoxin (6). The coating of phospholipids separating the inner solution (rich in pyridoxin) from the depleted outer vacuolar solution could be easily demonstrated by the molybdenic reagent.

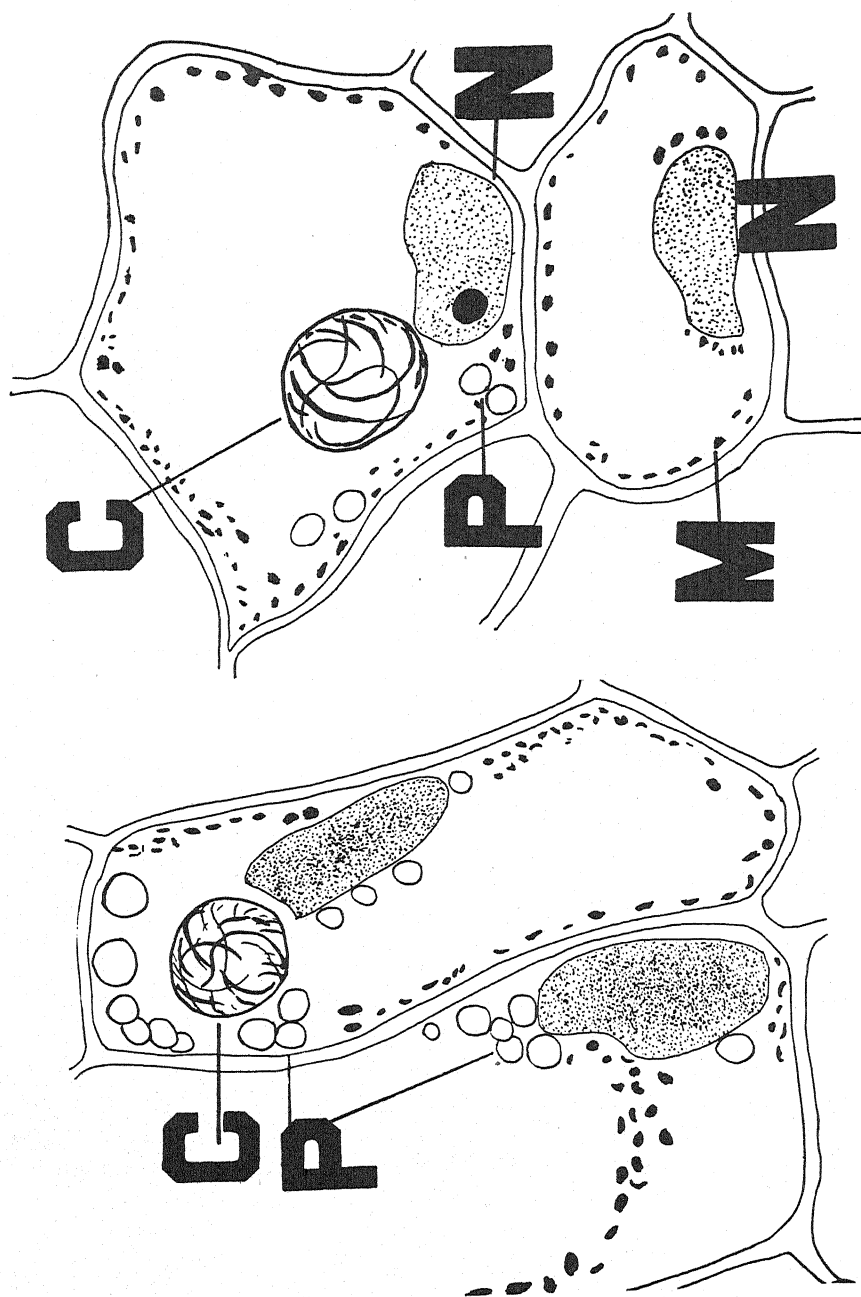


FIG. 1. Cells from a stalk of sugar cane infiltrated with a solution of sodium xanthogenate. These sections were treated with the molybdenum reagent, revealing the nucleoproteins in the nucleus, N, in the plastids, P, and the mitochondria, M, and also the phosphatides at the periphery of the coacervates, C.

These coacervates could also be demonstrated in permanent mounts of sections of tissues fixed in the HELLY's killing fluid, and properly stained with haematoxylin or with acid fuschsin. Ultimate stages of cell disintegration in sugar cane, either due to physiological senescence, or pathological breakdown are expressed by the prevalence of coacervates which may eventually be the only recognizable features. In fact the intermediate phase of the coacervate is so permanent a structure that the inner core of phenolic

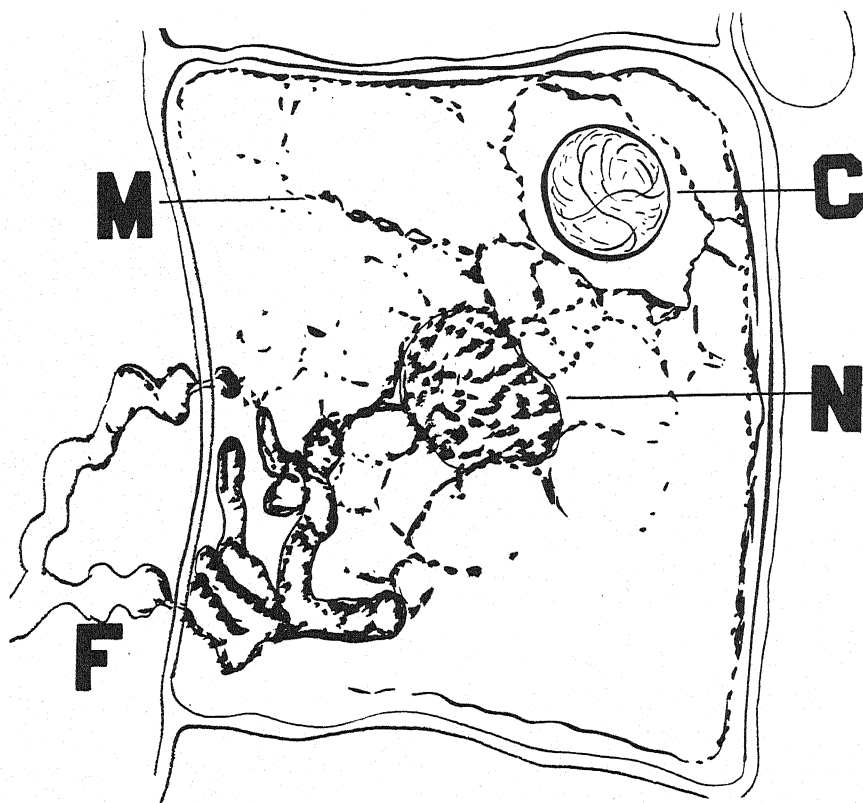


FIG. 2. A cell from stalk of sugar cane penetrated by hyphae, F, of *C. falcatum* fixed in HELLY fluid and stained with haematoxylin. The nucleus, N, at the center of the cell shows numerous prochromosomes; mitochondria, M, are lined up between a number of vacuoles. The segregated vacuole in the upper right corner contains a large coacervate, C, outlined by the deeply stained phospholipoid envelope.

compounds can be dissolved out by proper solvents (for instance tetra ethylene glycol or methyl ethers), leaving the envelope intact as an empty shell.

Whereas coacervates are constantly present in nodes of young canes affected by chlorotic streak, they generally do not occur in homologous tissues of young, actively growing, healthy canes, but they make their appearance later, toward the end of the growth cycle, about the time for harvest. They also appear in cells affected by various diseases besides chlorotic streak, and

they can be made to appear at will, following injection of the stalks by dilute solutions of agents acting as inhibitors of some respiratory systems, for instance salicylaldoxime (10 to 100 p.p.m.) or sodium xanthogenate (100 p.p.m.).

Coacervates similar to those observed in chlorotic streak also occur in cells of cane infected by *Colletotrichum falcatum*, the pathogenic fungus responsible for the Red Rot disease (fig. 2). The disease is characterized by a discoloration due to anthocyanol formation in the vacuolar solution of cells adjoining the area of infection. Conidia of the fungus travel upwards and downwards along the pitted vessels from the point of inoculation, having a tendency to lodge and germinate a short distance below the nodes. The germ tubes which grow through pits into the adjoining slender, elongated parenchyma cells cause the vacuolar solutions to be dispersed into a number of vacuoles possessing high oxidase activity, and soon producing the above-mentioned red pigment. Some of this oxidized, polymerized material oozes from the vacuoles through pits in the wall into tyloses. That reaction efficiently blocks any further progress of the germ tubes in resistant canes. In susceptible canes, however, the germ tubes grow as hyphae from one parenchyma cell to the next, or in the intercellular spaces, inducing non-compensated respiration in cells as much as five or six rows distant, as evidenced by the oxidation of paraphenylene diamine hydrochloride to the red quinoid derivative (Würster red) in the vacuolar solutions. The red anthocyanol pigment formed in the parenchyma cells of susceptible cane oozes from the vacuoles into the intercellular spaces and coacervates may appear in some vacuoles. In Red Rot tissues we therefore witness both the external secretion of vacuolar material, even to the intercellular spaces, and also that peculiar type of "internal secretion" which results in coacervate formation.

Cell inclusions in the vine disease, now called "Pierce's disease," were observed by VIALA and SAUVAGEAU in 1892 (15) and then misinterpreted as plasmodia of a Myxomycete. DUCOMET (5) abandoned the erroneous idea of plasmodial parasites and gave a cytochemically correct interpretation of the small and large spherical bodies in the vacuolar sap, opening the way to the recognition of the prevalence in other plants of those phenolic or tannic compounds which may become conspicuously coacervated or otherwise polymerized (12). Pierce's disease is characterized physiologically by the failure of internodes to elongate and cytochemically by coacervation of phenolic compounds. A similar correlation between dwarfed shoots and coacervation was demonstrated as the result of zinc deficiency in apricots and walnuts (REED and DUFRENOY) (13).

Cytochemical examination of grapes experimentally infected in the greenhouses of the University of California by Dr. J. H. FREITAG demonstrates the prevalence of coacervates in the perivascular cells of the stems. A longitudinal section after proper fixation in the HELLY's killing fluid and staining with haematoxylin, showed distorted nuclei (some with amyloplasts

closely appressed) and one or several coacervates sharply outlined. In contiguous cells the phenolic material was often flocculated in the vacuole, and many times could be distinguished as a mass of small spherical drops. In other cases the vacuolar space was almost filled with a diffuse material giving the chromaffinic reaction.

#### CYTOCHEMICAL REACTION

Coacervates in the vacuolar solution appear as features in a three phase system. The inner phase may be defined as a "gelatinous hydrate" of phenolic compounds and the outer phase as the depleted vacuolar solution. The intermediate phase is made of amphoteric phospholipids. The identification of coacervates therefore calls for the cytochemical localization of the phenolic compounds and of the phospholipids.

#### PHENOLIC COMPOUNDS

The cytochemical reactions may range from those indicative of some active C-OH group or dienol HO-C-C-OH or paraphenol, to those of highest specificity for some definite configuration of the ring structure or active groups.

The reactions resulting in the formation of bright azo-dyes have been shown by LISON to grade from the general localization of phenolic compounds to the specification of definite compounds, according to the cytochemical technique applied. The diazo-reaction, which SWAMINATHAN (14) used for the quantitative estimation of vitamin B<sub>6</sub> may also be used for the detection of pyridoxin in coacervates, or in sections of fresh plant material. A convergent line of evidence as to the presence of pyridoxin in coacervates may be obtained by simply immersing freehand sections of tissues in a suspension of 2.6 dichloroquinone chloroimide buffered at pH 8.8 with sodium borate (6).

#### DEPLETED VACUOLAR SOLUTIONS

The vacuolar solution in which coacervates are floating is evidently depleted in dispersed materials which would respond to vital staining, and concomitantly depleted in PO<sub>4</sub> ions, since the phenolic compounds apt to absorb the vital dyes have become coacervated in the inner phase of the coacervate, and the ionic PO<sub>4</sub> has entered into combination with lipids to form the third or intermediate phase of the auto-complex coacervate.

Sections of tissues at the site of non-compensated respiration may show cells where coacervates are sharply outlined from the otherwise empty vacuole, in contrast to the neighboring cells, where phenols have not been coacervated as yet, and whose vacuolar solution stains as a whole with vital dyes, or responds to the chromaffin reaction.

#### THIRD (INTERMEDIATE) PHASE

This phase may be identified as to either of its constituents: The phosphorus being responsible for the formation of molybdenum blue with

molybdenum reagent, and the fatty materials staining solid black a few minutes after the section has been immersed in the hydrated methylal solution of Sudan Black (7). The intermediate phase represents a solid physical structure, which persists as an empty shell after the phenolic contents have been dissolved out by proper solvents such as polyethylene glycol or by methyl ethers. In healthy cells the  $\text{PO}_4$  ions from the vacuolar solution are used almost as fast as they become available for the building up of nucleic acids and other energy-rich phosphorus compounds through esterification. In hypoplastic cells, coacervation ties up phosphorus in the solid auto-complex structure, thus making it unavailable for phosphorylation of carbohydrates in cell metabolism, which may account for the dwarfed shoots characteristic of rosette of fruit trees or the "Court Noué" of vines.

It remains for future investigations to show how the activity of the enzymic systems may be influenced by the alterations in equilibria due to the formation of auto-complex coacervates in cell vacuoles.

### Summary

1. The study of coacervation in hypoplastic cells demonstrates some important correlations with the respiratory activities in the vacuoles.

2. Auto-complex coacervates appear as refringent spherical bodies in the vacuoles of hypoplastic cells affected by virus, or parasitized by certain fungi, or deficiency of some essential microelement. They have also been induced in tissues treated with compounds tending to block some component of the cell respiratory system.

3. The data presented show that these refringent spherical bodies result from the coacervation of phenolic compounds from the vacuolar solution concomitant with the adsorption of phosphatides at the interphase between the coacervated compounds and the water phase of the vacuole. Both physico-chemical phenomena are related to a shift of the respiratory systems toward a higher rH.

4. Coacervation is one of the several ways whereby a phase originally homogeneous may become differentiated into several. Coacervation of phenolic materials within the vacuole does not imply any change of cytoplasmic permeability (although the same disorders in cell respiration which induce coacervation may enhance permeability).

A portion of the work herein reported was accomplished while the senior author was an exchange professor at the University of Louisiana.

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CONCERNING THE FUNCTION OF RUBBER HYDROCARBON  
(CAOUTCHOUC) IN THE GUAYULE PLANT, *PARTHENIUM*  
*ARGENTATUM* A. GRAY<sup>1</sup>

HAMILTON P. TRAUB

(WITH FOUR FIGURES)

LLOYD (11, 12), who originally believed that rubber hydrocarbon (caoutchouc) in the guayule plant occurred in the parenchymatous cells as solid particles, stated that "rubber appears to have no physiological function in the guayule plant"; but this viewpoint was questioned by SPENCE (21) on the basis of work with oxydases in *Hevea* latex. Later LLOYD (13) demonstrated that the rubber in the parenchymatous cells of guayule "occurs as a colloidal suspension, precisely as in the latex of *Hevea*," and this led him to modify his viewpoint for he realized the possibility that rubber in colloidal suspension could be attacked by enzymes. In support of this hypothesis could be cited the work of FAIVRE (2, 3, 4), with *Tragopogon porrifolius*, *Morus alba*, and a *Ficus* sp., who varied the conditions, including absence of carbon dioxide, under which they were grown, and observed impoverishment of the latex; and the experiment of SPENCE (21) who reports that young *Ficus elastica* "grown in an atmosphere and soil free of carbon dioxide, gradually drew on their milk which became nothing more than water after a few weeks' time." However, LLOYD (13) insisted that proof was required for the hypothesis that the rubber hydrocarbon in guayule is actually a reserve substance and is made use of in metabolism.

The results of SPENCE and MCCALLUM (22) showed a loss in grams of rubber per plant during conditions that favored utilization of carbohydrate reserves. However, observations during the course of experiments from 1942 to 1944 showed no such loss of rubber occurring in guayule plants under similar conditions. It was thought possible because of the great variability of seedling guayule plants that the results of SPENCE and MCCALLUM (22) may have been vitiated by failure to adequately replicate the treatments in order to eliminate the possibility of the differences being nothing more than chance variation. Since this subject is very important not only from the theoretical standpoint of plant physiology but also in connection with guayule rubber production, the work here reported was undertaken to test the conclusions of previous workers.

### Methods

Guayule nursery seedlings were grown in gravel culture in the greenhouse under three levels of carbon assimilation:

Treatment I. No leaves removed.

<sup>1</sup> Contribution from the Emergency Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Treatment II. All leaves removed at the beginning of the experiment.

Treatment III. All leaves removed at the beginning of the experiment, and new top growth at intervals after growth began.

The very low level of carbon assimilation in treatment III was calculated to exhaust the carbohydrate reserves in the plant in order to test the hypothesis that rubber hydrocarbon (caoutchouc) may be used as a food.

The 1500 plants required for the experiment were selected for uniformity from more than 20,000 field run plants that had been seeded in June, 1943, on Chualar loam soil at the Alisal Nursery of the Emergency Rubber Project, Salinas, Calif., and were therefore 16 months old when the plots were set up in October, 1944. The roots were cut uniformly to 6 inches. In physiological studies, the results are usually expressed on the basis of total dry weight which is a changing basis when the developing plant is involved. MASON and MASKELL (14) have suggested the residual dry weight as an improvement for it is less subject to change. In the present experiment, the rubber, resins and carbohydrate results are expressed on the basis of constituents, in grams, in relatively constant regions: The original (a) 6-inch (15.24 cm.) root with crown diameter of  $6.4 \pm 0.066$  mm., and (b) stems with  $5 \pm 0.12$  branches; height  $16.6 \pm 0.206$  cm. In addition, the new roots, and new growth were analyzed for rubber, resins, and carbohydrates.

The plants were grown in gravel culture in  $\frac{1}{2}$ -gal. glazed crocks provided with a hole for drainage on one side near the bottom. Three plants were grown in each crock, and five such crocks with plants, 15 plants in all, constituted the unit plots for sampling purposes. Each treatment for each of the three sampling dates was randomly replicated ten times. The plants were given Hoagland's nutrient solution No. 1, supplemented with boron, manganese, zinc, copper, molybdenum and iron as recommended by HOAGLAND and ARNON (9) and flushed out with tap water on alternate days. The night temperature in the greenhouse was kept at approximately 75° F., and the day temperature ranged between 80° and 90° F.

All of the plots were sampled at the beginning of the experiment on Oct. 21, 1944, and again on Dec. 19, 1944, and Jan. 19, 1945. The fourth sampling for treatment III was made on Feb. 15, 1945.

The plants, with ball of gravel containing any new roots, were carefully removed by tipping the top of the crocks downward, and the new roots with gravel were held in a tub of water thus allowing the loose gravel to sink to the bottom. When all 15 plants constituting the particular unit plot had been treated in this manner, the new root portions left behind in the tub of water were recovered according to the method described by TRAUB (25) and added to the new root fraction of the plot. At the first sampling, the plants were separated into three portions: tap roots, branches, and leaves. Thereafter two portions were added; new roots and new top growth.

The various fractions were separately dried in a mechanical convection oven at 65° C., for 48 hours, and after weighing for the determination of total dry weight, were finely ground according to the procedure described

by TRAUB (24) for rubber, resins and carbohydrate analyses. It should be noted that the new roots fraction still contained any adhering gravel after drying, but this was removed before the tissue was ground, according to the procedure described by TRAUB (25).

After the experiment was started, new top growth was removed from plants of treatment III at intervals (Nov. 20, Dec. 5, Dec. 19, Jan. 9, Jan. 19, and Feb. 15) in order to keep the assimilation of carbon at a relatively low level. The new top growth removed at intervals between regular sampling dates was included with the dry weight and the tissues analyzed for the various constituents at the regular sampling dates.

Rubber and resins were determined according to the photometric procedure of TRAUB (24). Total water soluble carbohydrates, free sugars, levulins, inulin, and the relative amounts of 89 per cent. ethanol insoluble and 89 per cent. ethanol soluble levulins were analyzed according to the procedure described by TRAUB and SLATTERY (26), and pentosans according to the method of MCRARY and SLATTERY (15). The data have been subjected to appropriate statistical analysis, in most cases by the use of FISHER's analysis of variance (7, 5, 6, 20).

### Data

#### GROWTH RESPONSES

Plants not defoliated (treatment I) showed a lag in growth resumption after the experiment was set up in the greenhouse. After two months, on



FIG. 1. Growth responses of guayule plants as affected by three levels of carbon assimilation: 1. Not defoliated; 2. Once Defoliated; 3. Defoliated and new growth repeatedly removed. Upper, at end of period, Oct. 21 to Dec. 19, 1944; and lower, at end of period, Dec. 20, 1944, to Jan. 19, 1945.

Dec. 19, 1944, only 78.6 per cent. of the plants under treatment I had sprouted. In contrast, similar plants when defoliated (treatments II and III) sprouted promptly after planting. These results are in harmony with those reported by SMITH (18, 19) and ERICKSON (1).

The plants in treatment I, with no leaf removal, soon appeared somewhat shriveled and apparently were suffering from excessive moisture loss through transpiration, an effect similar to that reported by ERICKSON (1). By Jan. 19, all of the plants in treatment I had resumed growth, but there was variation in degree of development as shown in figure 1. Such variation in growth responses was apparently due to the injurious effect on plant tissues when there was a moisture deficit in the plants for a time after the experiment was started in October.

TABLE I

EFFECT OF DEFOLIATION ON MEAN DRY WEIGHT, IN GRAMS PER PLOT, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944-1945

PLANT ORGAN	SAMPLING DATES	WEIGHT PER PLOT†			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTER-ACTIONS*	
					0.05	0.01	0.05	0.01
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
Original leaves and new top growth‡	Oct. 21	15.67	15.72	15.33	1.64	2.20	2.32	3.11
	Dec. 19	18.70 (+ 3.03)§	13.47	6.45				
	Jan. 19	32.36 (+ 16.69)	30.25	9.61				
Original stem and branches	Oct. 21	47.38	45.62	47.09	2.52	3.56	3.38	4.78
	Dec. 19	45.25	39.84	38.33				
	Jan. 19	40.28	40.93	35.89				
Original 6" roots	Oct. 21	24.72	24.44	24.43	1.42	1.91	2.01	2.70
	Dec. 19	23.80	24.52	21.74				
	Jan. 19	24.74	25.23	20.10				
New roots	Dec. 19	0.47	1.72	0.72	0.55	0.75	0.78	1.06
	Jan. 19	5.00	8.35	1.24				

\* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original dry weights of leaves.

In the case of plants defoliated at the beginning of the experiment only (treatment II), growth resumption was rapid and total growth of tops and roots was superior to plants of the other two treatments (I and III) as shown in figure 1.

Plants of treatment III, defoliated at the beginning of the experiment, followed by removal of new top growth at intervals thereafter, started out exactly like the plants in treatment II, but after each defoliation there was

a lag in growth followed by renewed sprouting and growth. The total effect of the repeated removal of new top growth was distinctly shown in the relatively poor root development as indicated in figure 1. In a few cases, toward the end of the experiment (after Jan. 19), the tips of the branches died back for  $\frac{1}{4}$  to  $\frac{1}{2}$  inch, but such dying back did not affect the rubber content as will be shown below.

The dry weight data on the basis of grams per plot are summarized in table I, together with analyses of variance.

In connection with the subject of changes in total dry weight it will be of interest to consider the amount of dry matter lost to the guayule plant,

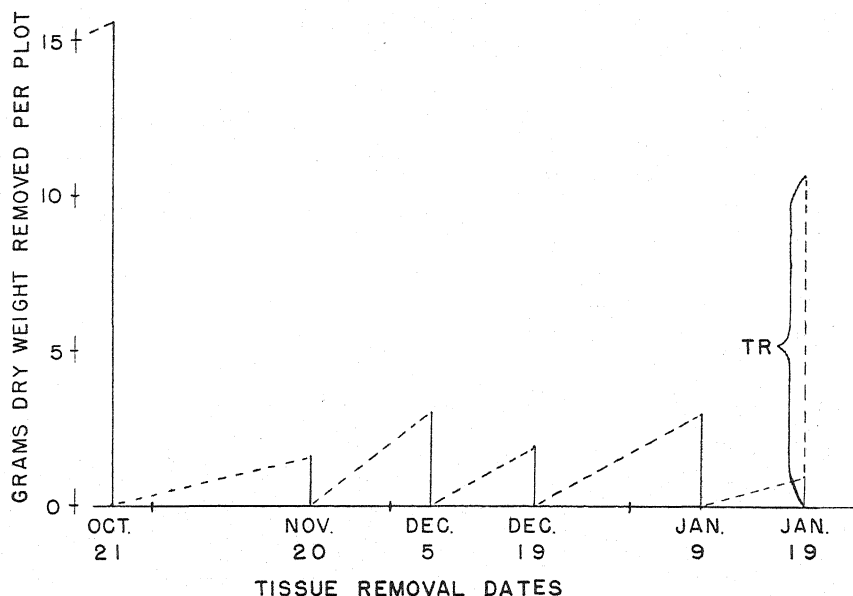


FIG. 2. Amount of dry weight, in grams, removed per plot from guayule plants in treatment III by repeated defoliations from Oct. 21, 1944, to Jan. 19, 1945. Solid vertical lines represent grams dry weight removed per plot on harvesting dates indicated; line enclosed by bracket, TR, represents total new top growth removed per plot after initial defoliation on Oct. 21.

in treatment III, by the repeated removal of new growth. The data are shown graphically in figure 2. This shows that 15 grams of dry matter per plot were removed at the beginning of the experiment, and that during the following three and a half months, 10 grams of dry matter were lost to these plants due to removal of new growth. In contrast, the increase in dry matter per plot due to new growth, for treatments I and II, was not lost to the plants. From the standpoint of total grams of dry matter produced irrespective of its removal, plants of treatment I, not defoliated, laid down approximately half as much dry weight per plot (16.5 gm.) as plants of treatment II, once defoliated (30.3 gm.); and plants of treatment III, repeatedly defoliated, produced only about one-third as much (9.6 gm.) per plot.



The original stems and branches lost dry weight in all cases, but the loss in plants of treatment III, repeatedly defoliated ( $-11$  gm. per plot) was greatest. Roots showed a loss in dry weight only for plants of treatment III, repeatedly defoliated ( $-4.7$  gm.). The marked losses of dry weight per plot in stems and roots for this severe treatment demonstrate the effectiveness of the technique of repeated removal of new top growth for starving plants.

The increase in dry weight per plot due to new root growth was greatest for plants of treatment II, once defoliated ( $8.4$  gm.), and lowest for plants of treatment III, repeatedly defoliated ( $1.34$  gm.).

#### CHANGES IN CARBOHYDRATES

The consideration of changes in carbohydrate reserves is particularly important in evaluating the effect of very low carbon assimilation (treatment III) on the functioning of the plant. The carbohydrate analyses of stems and roots are briefly summarized in tables II and III, and figure 3. The changes in carbohydrates, in most cases, show similar trends whether they are considered on the percentage dry weight, or grams per plot basis. Since the latter gives a truer approximation of the trends, the data are here briefly considered on that basis.

Free sugars of stems and roots remain practically constant for plants under treatment I from Oct. 21 to Dec. 19, but decline for treatment II and III. In the latter case they decrease to almost 0. From Dec. 19 to Jan. 19, the free sugars in stems and roots of plants under treatment II rise to the original level, but remain at almost 0 for treatment III.

The levulins in stems and roots decrease for treatments I and III from Oct. 21 to Dec. 19, reaching almost 0 for treatment III. For treatment II there is an abrupt drop ( $P = 0.01$ ) from Oct. 21 to Dec. 19, followed by a slight rise ( $P = 0.05$ ) from Dec. 19 to Jan. 19.

The inulin of stems and roots declines for all treatments but the decrease is at a slower rate for treatment I for the low is reached for treatments II and III by Dec. 19 whereas a similar low is not reached for plants of treatment I until Jan. 19. Again the drop for treatment I is almost to 0.

The changes in total water-soluble carbohydrates for the three treatments show the same tendencies as those of the levulins which make up the greatest proportion of the whole.

Similarly the 89 per cent. ethanol soluble levulins showed the same tendencies as the levulins, but the 89 per cent. ethanol insoluble levulins followed trends similar to those shown by the inulin contents.

The pentosans of stems show a decline for all treatments from Oct. 21 to Jan. 19, but the decrease is greatest for plants under treatment III. The pentosan content of roots did not change perceptibly from Oct. 21 to Jan. 19, except for a decrease ( $P = 0.01$ ) for plants under treatment III by Jan. 19.

These results show conclusively that the plants under treatment III, repeatedly defoliated, were so effectively starved on account of the very low level of carbon assimilation that the water-soluble carbohydrate content—

TABLE II

EFFECT OF DEFOLIATION ON MEAN PERCENTAGE CARBOHYDRATES, OF PLANT ORGANS  
AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE,  
SALINAS, CALIFORNIA, 1944-1945

PLANT ORGAN	SAMPLING DATES	PERCENTAGE DRY WEIGHT			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLI- ATED	ONCE DEFOLI- ATED	REPEAT- EDLY DEFOLIATED	MEANS*		INTER- ACTIONS*	
					0.05	0.01	0.05	0.01
		%	%	%	%	%	%	%
FREE SUGARS								
Stems†	Oct. 21	0.54	0.53	0.55	0.06	0.08	0.08	0.11
	Dec. 19	0.65	0.34	0.20				
	Jan. 19	0.57	0.59	0.20				
Roots†	Oct. 21	0.58	0.57	0.59	0.08	0.11	0.11	0.16
	Dec. 19	0.57	0.43	0.25				
	Jan. 19	0.53	0.62	0.14				
LEVULINS								
Stems	Oct. 21	11.35	11.39	11.28	0.67	0.89	0.95	1.26
	Dec. 19	5.58	0.93	0.52				
	Jan. 19	2.44	1.70	0.19				
Roots	Oct. 21	14.51	14.75	14.64	0.73	0.98	1.03	1.39
	Dec. 19	8.14	2.19	1.07				
	Jan. 19	5.16	4.41	0.30				
INULIN								
Stems	Oct. 21	1.28	1.19	1.22	0.33	0.44	0.47	0.62
	Dec. 19	0.78	0.20	0.22				
	Jan. 19	0.70	0.30	0.21				
Roots	Oct. 21	2.13	2.20	2.21	0.47	0.64	0.66	0.90
	Dec. 19	1.40	0.52	0.48				
	Jan. 19	0.77	0.45	0.13				
TOTAL WATER-SOLUBLE CARBOHYDRATES								
Stems	Oct. 21	13.18	13.11	13.16	0.77	1.04	1.09	1.47
	Dec. 19	7.00	1.48	0.94				
	Jan. 19	3.71	2.60	0.61				
Roots	Oct. 21	17.21	17.52	17.56	0.75	1.01	1.06	1.48
	Dec. 19	10.21	3.14	1.79				
	Jan. 19	6.47	5.48	0.56				
89 PER CENT. ETHANOL SOLUBLE LEVULINS								
Stems	Oct. 21	3.00	2.73	2.87	0.70	0.94	0.99	1.33
	Dec. 19	1.49	0.67	0.36				
	Jan. 19	1.42	1.69	0.22				
Roots	Oct. 21	3.16	3.41	3.29	0.69	0.92	0.98	1.30
	Dec. 19	2.86	1.34	0.61				
	Jan. 19	3.14	3.89	0.22				

TABLE II.—(Continued)

PLANT ORGAN	SAMPLING DATES	PERCENTAGE DRY WEIGHT			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEAT-EDLY DEFOLIATED	MEANS*		INTER-ACTIONS*	
					0.05	0.01	0.05	0.01
		%	%	%	%	%	%	%
80 PER CENT. ETHANOL INSOLUBLE LEVULINS								
Stems	Oct. 21	9.64	9.75	9.84	0.71	0.95	1.00	1.34
	Dec. 19	4.87	0.47	0.38				
	Jan. 19	1.72	0.32	0.18				
Roots	Oct. 21	13.48	13.53	13.68	0.66	0.88	0.93	1.24
	Dec. 19	6.79	1.37	0.93				
	Jan. 19	2.75	0.84	0.26				
PENTOSANS								
Stems	Oct. 21	2.52	2.52	2.50	0.08	0.10	0.11	0.16
	Dec. 19	2.19	2.10	2.05				
	Jan. 19	2.21	2.24	1.79				
Roots	Oct. 21	1.23	1.23	1.24	0.08	0.11	0.11	0.16
	Dec. 19	1.22	1.22	1.24				
	Jan. 19	1.32	1.39	1.15				

\* If values for treatments differ by more than the figures given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

free sugars, levulins and inulin—declined almost to 0 from Oct. 21 to Jan. 19. Even the pentosan reserves, not so readily available, were drawn upon due to the starved condition of the plants for the pentosan content of stems declined ( $P=0.01$ ) from 2.50 to 1.79 per cent., or from 1.18 to 0.64 grams per plot. Similarly the pentosans in roots declined ( $P=0.05$ ) from 1.24 to 1.15 per cent., or ( $P=0.01$ ) from 0.30 to 0.23 grams per plot. This is also the first concrete demonstration that pentosans in guayule, particularly in the stems, function as food reserves.

In figure 3 are shown graphically any gains and losses in total dry weight over the original weights, and the carbohydrate reserves (free sugars, levulins, inulin and pentosans) considered above. In the case of guayule stems the losses in dry weight for treatments I and III are greater than the losses in the reserve carbohydrates determined. In the roots there is a slight gain in dry weight for treatment II, no net loss for treatment I, and a decided loss in dry weight for treatment III. The losses in reserve carbohydrates for treatments I and II are not counter balanced by similar changes in dry weight as is the case for treatment III.

This suggests the possibility that in the stems still other compounds in the plant, such as pectins, may serve as carbon reserves. It is apparently necessary for the physiologist to go farther than the usual routine analyses in order to arrive at an adequate understanding of plant functioning.

TABLE III

EFFECT OF DEFOLIATION ON MEAN CARBOHYDRATE CONTENT, IN GRAMS PER PLOT, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944-1945

PLANT ORGAN	SAMPLING DATES	WEIGHT PER PLOT			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEAT-EDLY DEFOLIATED	MEANS*		INTER-ACTIONS*	
					0.05	0.01	0.05	0.01
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
FREE SUGARS								
Stems†	Oct. 21	0.25	0.24	0.26	0.03	0.04	0.04	0.06
	Dec. 19	0.29	0.14	0.08				
	Jan. 19	0.23	0.24	0.07				
Roots†	Oct. 21	0.15	0.14	0.15	0.02	0.03	0.03	0.04
	Dec. 19	0.14	0.11	0.06				
	Jan. 19	0.13	0.16	0.03				
LEVULINS								
Stems	Oct. 21	5.38	5.21	5.31	0.42	0.57	0.59	0.81
	Dec. 19	2.53	0.37	0.20				
	Jan. 19	0.98	0.70	0.07				
Roots	Oct. 21	3.58	3.61	3.61	0.22	0.29	0.31	0.41
	Dec. 19	1.91	0.54	0.24				
	Jan. 19	1.28	1.11	0.06				
INULIN								
Stems	Oct. 21	0.60	0.54	0.57	0.15	0.20	0.21	0.28
	Dec. 19	0.36	0.08	0.08				
	Jan. 19	0.28	0.13	0.08				
Roots	Oct. 21	0.53	0.54	0.54	0.12	0.16	0.17	0.23
	Dec. 19	0.34	0.13	0.10				
	Jan. 19	0.19	0.11	0.03				
TOTAL WATER-SOLUBLE CARBOHYDRATES								
Stems	Oct. 21	6.24	5.90	6.19	0.45	0.60	0.64	0.85
	Dec. 19	3.18	0.59	0.36				
	Jan. 19	1.49	1.07	0.22				
Roots	Oct. 21	4.26	4.28	4.29	0.23	0.31	0.33	0.44
	Dec. 19	2.39	0.78	0.40				
	Jan. 19	1.60	1.38	0.11				
89 PER CENT. ETHANOL SOLUBLE LEVULINS								
Stems	Oct. 21	1.41	1.25	1.35	0.33	0.44	0.47	0.62
	Dec. 19	0.68	0.27	0.14				
	Jan. 19	0.58	0.70	0.08				
Roots	Oct. 21	0.78	0.83	0.80	0.17	0.23	0.24	0.33
	Dec. 19	0.68	0.33	0.14				
	Jan. 19	0.78	0.98	0.04				

TABLE III.—(Continued)

PLANT ORGAN	SAMPLING DATES	WEIGHT PER PLOT			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEAT-EDLY DEFOLIATED	MEANS*		INTER-ACTIONS*	
					0.05	0.01	0.05	0.01
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
89 PER CENT. ETHANOL INSOLUBLE LEVULINS								
Stems	Oct. 21	4.57	4.46	4.64	0.43	0.58	0.61	0.82
	Dec. 19	2.28	0.19	0.15				
	Jan. 19	0.68	0.13	0.07				
Roots	Oct. 21	3.33	3.31	3.34	0.20	0.27	0.28	0.38
	Dec. 19	1.61	0.34	0.21				
	Jan. 19	0.68	0.22	0.04				
PENTOSANS								
Stems	Oct. 21	1.20	1.15	1.18	0.08	0.11	0.11	0.16
	Dec. 19	0.99	0.84	0.79				
	Jan. 19	0.89	0.92	0.64				
Roots	Oct. 21	0.30	0.30	0.30	0.03	0.03	0.04	0.04
	Dec. 19	0.29	0.30	0.27				
	Jan. 19	0.33	0.35	0.23				

\* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

#### CHANGES IN RUBBER CONTENT

The changes in rubber content are summarized in tables IV and V. In the former, the data are expressed in percentage of total dry weight, and in the latter in grams of rubber present in the regions, of new growth, original stems and branches, original 6-inch roots, and new roots.

In the case of original leaves and new top growth, by Dec. 19, on a percentage basis, a loss of 0.1 per cent ( $P = 0.05$ ) in rubber is indicated for treatment I, but on the basis of grams of rubber for the entire region no significant change is shown (+0.001 gm.). At the Jan. 19 sampling significant increases are indicated ( $P = 0.01$ ) on both the percentage dry weight and grams per region basis. Plants defoliated at the beginning of the experiment, treatments II and III, accumulated rubber, by Dec. 19, in the original leaves and new growth, and showed no increase ( $P = 0.01$ ) from treatment III, but a decided increase, from 0.05 gm. per plot by Dec. 19 to 0.16 grams on Jan. 19. It should be noted that the rubber content in this region either remained stationary or increased.

In the case of the original stems and branches, initial increases ( $P = 0.01$ ), on the percentage and regional bases, from Oct. 21 to Dec. 19 are shown for all treatments. From Dec. 19 to Jan. 19, a significant increase (0.01), on the percentage basis, for treatment I is shown, but no increases for treat-

ments II and III. However, on the grams per plot basis, no significant changes are indicated.

In the case of the original 6-inch roots, on the percentage basis, significant increases ( $P = 0.01$ ) in rubber content are indicated from Oct. 21 to Dec. 19, and from Dec. 19 to Jan. 19 for all treatments, excepting the period Oct. 21 to Dec. 19, for treatment II, in which case an increase is indicated only on the  $P = 0.05$  level. On the grams per plot basis, however, the situation is somewhat different. From Oct. 21 to Dec. 21, significant increases

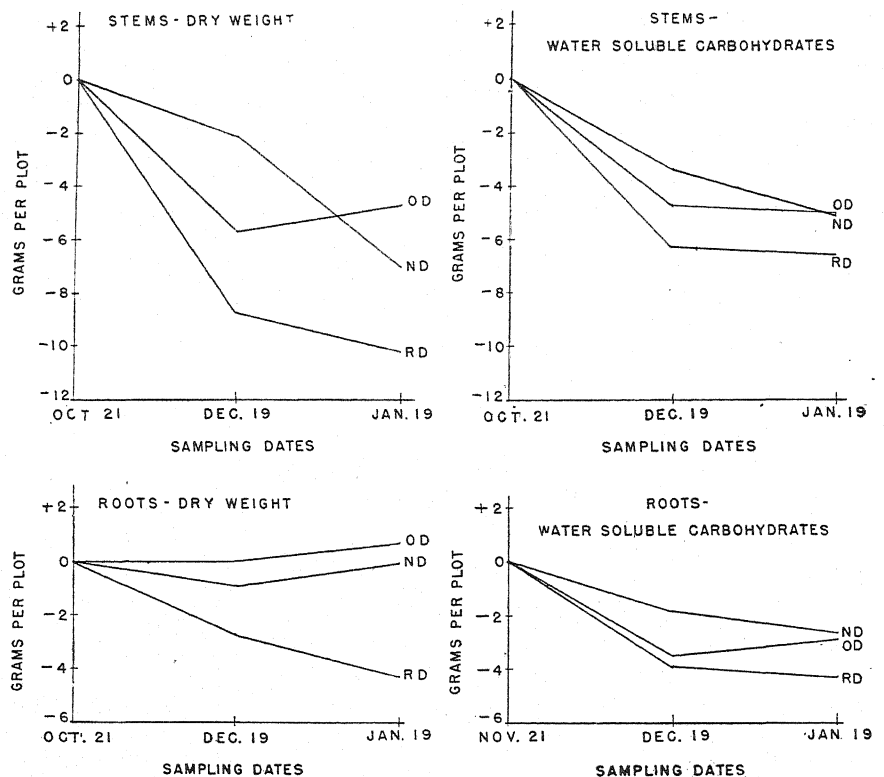


FIG. 3. Changes in dry matter and water-soluble carbohydrates (free sugars, levulins and inulin) of guayule plants, on grams per plot basis; treatments I (ND) not defoliated, II (OD) once defoliated, and III (RD) repeatedly defoliated.

( $P = 0.01$ ) are indicated only for treatments I and III and not for the rapidly growing plants in treatment II. From Dec. 19 to Jan. 19, however, the tendencies are reversed, for there is then a significant increase ( $P = 0.01$ ) for the normally developing plants of treatment II, and none for the retarded plants of treatments I and III.

A final harvest of plants under treatment III was made on Feb. 15. The percentages of rubber on this date for the original stems and roots were 10.86 and 11.08, respectively; and the corresponding grams of rubber per plot were 4.08 and 2.20, respectively, thus showing no significant changes in this constituent from Jan. 19 to Feb. 15.



TABLE IV

EFFECT OF DEFOLIATION ON MEAN PERCENTAGE RUBBER IN PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944-1945

PLANT ORGAN	SAMPLING DATES	PERCENTAGE RUBBER†			DIFFERENCE REQUIRED FOR SIGNIFICANCE		
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		
					0.05	0.01	INTERACTIONS*
Original leaves and new top growth‡	Oct. 21	% 0.641 0.541 (-0.100)§ 0.890 (+0.249)	% 0.635 0.398 0.553	% 0.639 0.322 0.346	gm.	gm.	gm.
	Dec. 19				0.076	0.102	0.107
	Jan. 19						0.144
Original stem and branches	Oct. 21	6.98 8.70 9.81	6.96 9.86 10.22	6.90 10.76 11.36	0.61	0.82	0.86
	Dec. 19						1.16
	Jan. 19						
Original 6" roots	Oct. 21	5.38 7.63 8.52	5.54 6.13 8.25	5.29 9.27 10.44	0.57	0.76	0.81
	Dec. 19						1.07
	Jan. 19						
New roots	Dec. 19	0.158 0.134	0.186 0.237	0.194 0.143	0.057	0.078	.....
	Jan. 19						

\* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original percentages.

TABLE V  
EFFECT OF DEFOLIATION ON MEAN AMOUNT OF RUBBER IN GRAMS PER PLOT, IN PLANT ORGANS AS INDICATED;  
GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944-1945

PLANT ORGAN	SAMPLING DATES	AMOUNT RUBBER PER PLOT†			DIFFERENCE REQUIRED FOR SIGNIFICANCE		
		Not DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTERACTIONS*
					0.05	0.01	
Original leaves and new top growth‡	Oct. 21	gm. 0.100 0.101 (+0.001)§ 0.285 (+0.185)	gm. 0.100 0.054 0.167	gm. 0.099 } 0.021 } 0.033 }	gm. 0.016	gm. 0.022	gm. 0.033
	Dec. 19						
	Jan. 19						
Original stem and branches	Oct. 21	3.32 3.92 3.94	3.19 3.93 4.13	3.25 } 4.12 } 4.08 }	0.34	0.45	.....
	Dec. 19						
	Jan. 19						
Original 6" roots	Oct. 21	1.33 1.82 2.11	1.36 1.50 2.08	1.29 } 2.01 } 2.10 }	0.19	0.25	0.35
	Dec. 19						
	Jan. 19						
New roots	Dec. 19	0.0007 0.0063	0.0032 0.0196	0.0014 } 0.0018 }	0.0035	0.0046	0.0067
	Jan. 19						

\* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original amounts present.

By Dec. 19 new roots had formed on all plants of treatments II and III, and on most of the plants in treatment I. From Dec. 19 to Jan. 19 no significant change in new roots could be shown even at the 0.05 level on a percentage of dry weight basis. On the basis of grams per plot significant increases ( $P = 0.01$ ) are shown for plants of treatments I and II, but none for treatment III.

These data show that the use of the percentage of dry weight basis in expressing the experimental results gave a somewhat different picture from that presented when results were expressed as grams present in a region of the plant. When results were expressed on the grams per region basis the rubber content invariably either remained constant or showed increases from one sampling date to the next so that no losses in rubber content are indicated.

The data show that growth and rubber accumulation in guayule tissues are not necessarily antagonistic since rubber increases were observed even during the period when plants resumed growth. It should be emphasized that the statement of LLOYD (12) that the rate of rubber secretion varies inversely with the rate of growth has been substantiated by the work of KELLEY, HUNTER and HOBBS (10) and others. The present data do not contradict any such principle for it concerns rate of rubber accumulation.

TABLE VI

EFFECT OF DEFOLIATION ON MEAN PERCENTAGE RESINS, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944-1945

PLANT ORGAN	SAM-PLING DATES	PERCENTAGE DRY WEIGHT†			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTER-ACTIONS*	
					0.05	0.01	0.05	0.01
		%	%	%	%	%	%	%
Original leaves and new top growth‡	Oct. 21	5.39	5.32	5.35	0.38	0.51	.....	.....
	Dec. 19	4.83 (-0.66)§	4.21	4.66				
	Jan. 19	4.74 (-0.65)	4.44	4.82				
Original stem and branches	Oct. 21	8.60	8.63	8.40	0.53	0.71	0.75	1.01
	Dec. 19	5.63	5.09	6.02				
	Jan. 19	6.01	5.95	5.74				
Original 6" roots	Oct. 21	8.69	9.01	9.11	0.48	0.64	0.67	0.90
	Dec. 19	6.82	6.04	6.18				
	Jan. 19	5.67	5.20	5.41				
New roots	Dec. 19	1.70	1.55	1.49	0.30	0.41	.....	.....
	Jan. 19	3.07	3.28	2.20				

\* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original dry weights of leaves.

## CHANGES IN RESINS CONTENT

The changes in resins content for the various tissue fractions are summarized in tables VI and VII. In the former, the data are expressed in percentage of total dry weight, and in the latter in grams of resins present in the regions of new growth, original stems, original 6-inch roots, and new roots.

The resins content of new growth, on the percentage basis, from Dec. 19 to Jan. 19, shows no significant change for treatments I and III, but a significant increase is indicated for treatment II. However, on the basis of grains

TABLE VII

EFFECT OF DEFOLIATION ON MEAN AMOUNT OF RESINS, IN GRAMS PER PLOT, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944-1945

PLANT ORGAN	SAMPLING DATES	GRAMS PER PLOT†			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTER-ACTIONS*	
					0.05	0.01	0.05	0.01
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
Original leaves and new top growth‡	Oct. 21	0.85	0.83	0.82	0.083	0.111	0.117	0.157
	Dec. 19	0.90 (+ 0.05)§	0.57	0.30				
	Jan. 19	1.54 (+ 0.69)	1.34	0.46				
Original stem and branches	Oct. 21	4.07	3.95	3.95	0.27	0.36	0.38	0.51
	Dec. 19	2.54	2.03	2.31				
	Jan. 19	2.42	2.43	2.06				
Original 6" roots	Oct. 21	2.13	2.20	2.14	0.17	0.22	.....	.....
	Dec. 19	1.62	1.46	1.34				
	Jan. 19	1.40	1.28	1.09				
New roots	Dec. 19	0.01	0.03	0.01	0.004	0.005	.....	.....
	Jan. 19	0.15	0.27	0.03	0.029	0.039	.....	.....

\* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original dry weights of leaves.

of resins per plot, a significant increase is shown for treatments I and II, but not for treatment III.

In the case of original stems, from Oct. 21 to Dec. 19, there is a very significant decrease in resins content ( $P = 0.01$ ) on the basis of percentage dry weight and grams per plot. However, from Dec. 19 to Jan. 19, such a decrease ( $P = 0.01$ ) holds only for stems of treatment II.

There is a continued decrease in resins ( $P = 0.01$ ), for 6-inch roots, during the entire experiment, from Oct. 21 to Jan. 19, on the percentage and grams per plot bases, except that for roots of treatments I and II on a gram per plot

basis from Dec. 19 to Jan. 19, a decrease can be shown only on the  $P = 0.05$  level of significance.

New roots, from Dec. 21 to Jan. 19, for treatments I and II showed increases in resins, but this was not the case in treatment III where plants were grown under a very low level of carbon assimilation.

#### RELATION OF RUBBER AND RESINS CHANGES

In figure 4, the changes in rubber and resins contents for the various regions of the plant, on the basis of grams per plot, are plotted against time.

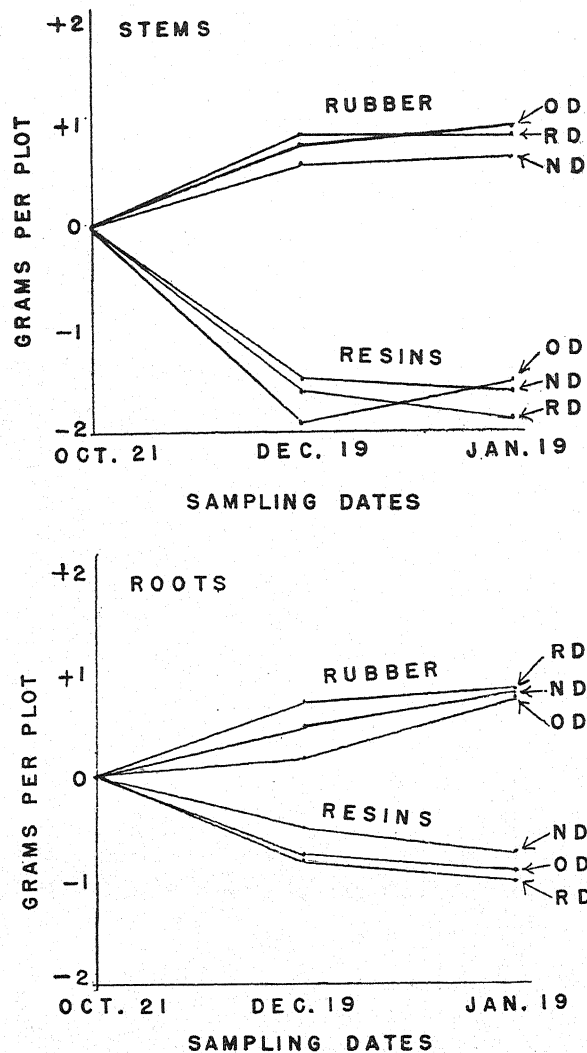


FIG. 4. Gain (+) or loss (-) in rubber or resins content of guayule stems and roots, on grams per plot basis; treatments I (ND) not defoliated, II (OD) once defoliated, and III (RD) repeatedly defoliated.

This shows unmistakably a negative correlation between changes in these two fractions in guayule tissue during the period when growth is resumed. It has already been pointed out (fig. 3) that the loss in dry weight of roots for treatment III, approximately equals that of the total carbohydrate fractions determined, but that in the stems, for treatment III, the loss in dry weight is greater than the latter total. Since the negative correlation shown in figure 4 is true for both roots and stems, it appears that during this period, the increase in rubber may be at the expense of some fraction or fractions of the resins (a mixture) functioning as rubber precursors. Other possibilities are considered in the succeeding section.

### Discussion

The results presented show above all that the technique devised for growing guayule plants under a very low level of carbon assimilation (treatment III) in comparison with plants grown under a normal level of carbon assimilation (treatment II), was effective in starving the plants to such an extent that the more readily available water-soluble carbohydrates were practically depleted, and even part of the pentosans were used up. MURNEEK (16) has reviewed the subject of the rôle of hemicelluloses in woody plants, including his own contributions, which indicate that these complex carbohydrates "are not merely aggregates of the plant cell wall but also serve as reserve substances." SPOEHR (23) suggested that hemicelluloses are primarily pentosans. The present results as shown in figure 3, indicate that in the guayule stem, in addition to the pentosans which are only partially used as food even under conditions of severe starvation, in treatment III, there are still other substances present that may serve as food reserves and which are more readily available than the pentosans. What these other substances are is not known but they might consist of pectins.

The data also show, that in spite of the fact that the plants were under this extreme food (carbon) deficit, the rubber content, on the basis of grams per plot, never decreased but rather increased during the course of the experiment. The function of rubber in guayule tissues, whatever it may be, does not appear to be that of a food reserve.

It may be that rubber performs some other function in the economy of the plant that has aided it in its struggle for survival. The guayule plant is one of the most efficient rubber factories ever devised by nature for, from the wild, strains have been selected that produce more than 18 per cent. of pure rubber hydrocarbon (caoutchouc) on the plant dry weight basis in a few years under cultivation. If rubber were merely an inert constituent serving no function in the economy of the plant, such a large amount of excess baggage in the cells might be a distinct handicap in the competitive struggle for existence. On the basis of the present data, however, we can only conclude that rubber apparently does not function as a reserve food in guayule. What other function, if any, it may perform in the plant is still an open question.



The fact that increases in rubber, under all treatments, were associated with decreases in resins content in the tissues of the plant, suggests the possibility that a constituent of the resins fraction might serve as a rubber precursor. It should be emphasized, however, that the resins fraction, a mixture, would include also any such compounds as essential oils and fats that might be present. Unfortunately, only a few of the constituents of the resins fraction have as yet been identified as shown by the summary of TRAUB (24), and, therefore, it is not possible to point to a compound, or compounds, that might be considered from the standpoint of rubber precursors. According to PROKOFIEFF (17) and HAAGEN-SMIT (8) such a precursor would more likely be an aliphatic than a cyclic compound.

### Summary

An experiment was conducted to determine the effect of carbon assimilation levels, ranging from very low to the normal, on the utilization of reserve foods in the guayule plant.

Guayule plants grown under a very low level of carbon assimilation by repeated removal of new top growth, (a) depleted their reserves of free sugars, levulins and inulin almost to 0; (b) utilized part of the pentosans; (c) lost no rubber hydrocarbon (caoutchouc), but showed a significant increase which was associated with a corresponding decrease in the resins fraction (a mixture), and (d) in the stems, lost a greater amount in dry weight than could be accounted for on the basis of loss in the water-soluble carbohydrates and pentosans determined.

The data show that: (a) the rubber hydrocarbon (caoutchouc) in the guayule plant apparently does not function as a food reserve, (b) the resins fraction (a mixture) may contain a rubber precursor, and (c) other reserves, such as pectins, might possibly be important food reserves and therefore deserve greater attention in physiological research.

The cooperation of the U. S. FOREST SERVICE is gratefully acknowledged.

BUREAU OF PLANT INDUSTRY  
SOILS AND AGRICULTURAL ENGINEERING  
U. S. DEPARTMENT OF AGRICULTURE  
BELTSVILLE, MARYLAND

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# THE EXTENSION OF PLANT ROOTS INTO DRY SOIL<sup>1</sup>

ALBERT S. HUNTER AND OMER J. KELLEY

(WITH TWO FIGURES)

In the course of extensive fertilizer and irrigation experiments with guayule, there arose the questions of the ability of plant roots to extend themselves into dry soil and to absorb nutrients from such soil. These questions are of fundamental interest and of considerable practical importance, particularly in the case of the latter. The greatest concentration of available nutrient elements is usually in the topsoil, which is the first soil to become dry. If plants are not able to use water that is available to some other part of the root system for the absorption of nutrients by roots in "dry" soil, in some cases the elements added to the soil as fertilizers may be unavailable to the plant.

The literature records disagreement as to the ability of plant roots to extend into soil having moisture content below the permanent wilting percentage. HENDRICKSON and VEIHMEYER (4) concluded that the roots of sunflowers will not grow into soil which contains less moisture than is present at the permanent wilting percentage. SHANTZ (8) believed that the roots of some drought-resistant plants are able to penetrate dry soil, but that ordinary crop plants lack that ability. MAGISTAD and BREZEALE (6) and BREZEALE (2) made extensive studies of the moisture equilibrium between soil and plant and concluded that not only will roots elongate into dry soil, but the plant can absorb water through roots in moist soil, transport the water, and build up the moisture content of a dry soil to the wilting percentage.

An experiment was carried out in 1945 to investigate the penetration of roots into dry soil in atmospheres of varying degrees of relative humidity, and to study the uptake of nutrient elements from dry soil by roots of which a part were in moist soil. Corn was used as the experimental plant, in the belief that the findings would apply to other plants.

## Experimental procedure and conditions

The photograph and schematic diagram of figure 1 show the arrangement of the component parts of the experimental apparatus. Sixteen pots of approximately 4-inch diameter and 8-inch height were prepared by brushing a mixture of equal parts of tar<sup>2</sup> (road oil) and paraffin (Parowax) on coarse cheese cloth, which was then shaped into cylinders with crimped-on bottoms. Examples of these pots are shown in figures 1 and 2. The walls and bottoms

<sup>1</sup> Special Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Salinas, California.

<sup>2</sup> The use of road oil as a component of this mixture was suggested by Dr. A. C. HILDRETH.

were approximately  $\frac{3}{16}$ -inch thick. Before use the pots were filled with water and tested several days for leaks. All were water-tight. In order to further test the impermeability of the tar-paraffin mixture to water three air-dry Bouyoucos blocks (1) were coated with approximately  $\frac{1}{4}$ -inch of the material and immersed in water at room temperature for two weeks. Throughout the test the resistances of the blocks were above 250,000 ohms, indicating that no water penetrated the coating.

The pots were filled with a moist greenhouse potting soil and surrounded with approximately  $1\frac{1}{2}$  inches of packed "dry" soil contained in wire mesh baskets lined with commodity cloth. One of the wire baskets with pot

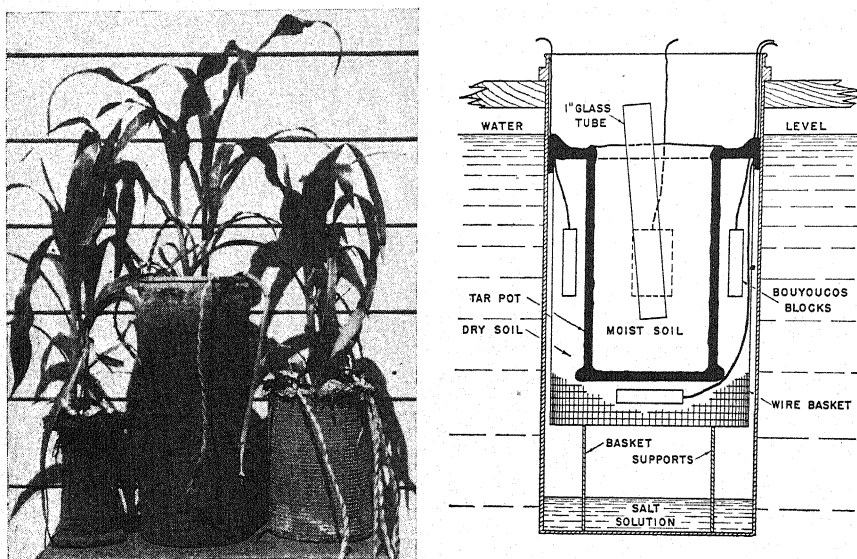


FIG. 1. Photograph and schematic diagram showing arrangement of component parts of experimental apparatus.

installed is shown in figure 1 (left). Approximately 4 kg. of air-dry Chualar loam, containing 0.4 per cent. moisture initially, was used with each pot. Its permanent wilting percentage and "field capacity" were approximately 4 and 12 per cent., respectively. With this soil was mixed approximately 0.5 kg. of dry quartz sand upon which a solution of radiophosphorus<sup>3</sup> had been dried. Four Bouyoucos blocks were spaced approximately equidistantly, in the air-dry soil, around the pot circumference, about midway between top and bottom and approximately  $\frac{3}{8}$  inch from the pot wall. A similar block was placed centrally beneath the pot and another was installed inside it.

The screen baskets were supported 4 inches from the bottom of steel

<sup>3</sup> The radiophosphorus (as sodium phosphate) was supplied to us through the kindness of DR. JOSEPH G. HAMILTON, Crocker Radiation Laboratory, University of California, Berkeley, California. The initial and final (after 30 days) quantities of radiophosphorus were approximately 17 and 3 microcuries per pot, respectively.

cans 18 inches tall. There was approximately  $\frac{1}{4}$  inch of free air space between the walls of can and basket. The rims of the pots were sealed to the walls of the cans by means of collars of cloth to which the tar-paraffin mixture was liberally applied. These seals confined the "dry" soil to the atmosphere in the space between the tar pot and the steel can. Four different conditions of relative humidity, about the "dry" soil surrounding the tar pots, were obtained through the addition of salt solutions to the bottoms of the steel cans, below the wire baskets. Each condition was replicated four times. These conditions are listed in table I.

To avoid fluctuations in relative humidity due to changes in temperature, the 18-inch steel cans were immersed to a depth of 15 inches in a constant-temperature tank which has been described by CAMPBELL and PRESLEY (3). Throughout the test period the temperature was maintained at  $84 \pm 2^\circ \text{F}$ . (except for one day, when a maximum of  $88^\circ \text{F}$ . was reached).

TABLE I

CONDITIONS OF RELATIVE HUMIDITY ABOUT THE "DRY" SOIL SURROUNDING THE TAR POTS

SERIES	POTS	ESTIMATED MAXIMUM RELATIVE HUMIDITY	ESTIMATED MINIMUM MOISTURE TENSION†	OBTAINED BY ADDING TO CAN
		PERCENTAGE	ATMOSPHERES	
A	3, 6, 12, 13	50	950	No solution
B	2, 5, 11, 16	82*	275	1 liter saturated NaCl (approx. 6 M)
C	1, 8, 10, 15	99*	15	1 liter 2.2 M NaCl
D	4, 7, 9, 14	100	0	1 liter water

\* Calculated from Raoult's Law,  $p = p_0 N_1$ .

† Calculated from the formula, tension in atmospheres =  $\frac{RT}{0.018} \ln \frac{100}{\text{relative humidity}}$ .

Three corn plants were grown in each pot. Water was added as required to the soil inside the pot to maintain a low moisture tension (resistance of Bouyoucos blocks around 500 ohms, or less). The resistances of the Bouyoucos blocks in the "dry" soil were determined at frequent intervals, usually two days, throughout the 30-day duration of the test.

The actual atmospheric conditions in the "dry" soil of the four series of pots were not determined. RUSSELL (6) indicates that the relative humidity of air-dry soil is about 50 per cent. The values of 82, 99, and 100 per cent. presented in table I are the highest values theoretically possible in equilibrium with the given solutions; it is very doubtful that values as these were actually reached during the test period. The estimated moisture tensions are the minimum values attainable in soil in equilibrium with the given maximum relative humidities.

### Results and discussion

Twelve days after germination of the corn, pots 14 and 15 were removed for examination. Several roots had penetrated the pot walls and extended



into the "dry" soil for about half an inch. The average moisture content of two separate samples of soil from the immediate vicinity of the roots was 1.04 per cent. for pot 14 and 0.80 per cent. for pot 15, as compared with an initial content of 0.4 per cent. The resistance of the Bouyoucos blocks was of the order of 35,000 ohms for pot 14 and 125,000 ohms for pot 15.

Pot 1 was examined after 24 days. Numerous roots had grown into the "dry" soil for one to two inches. The average moisture content of the "dry" soil about the roots was 1.12 per cent. and the average resistance of the Bouyoucos blocks was 31,000 ohms.

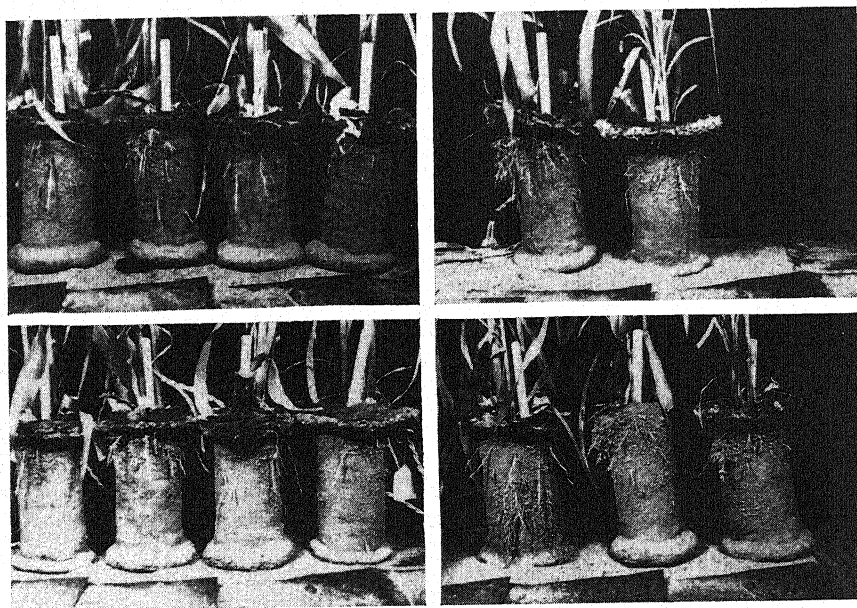


FIG. 2. Photographs showing corn roots that penetrated the walls of the tar pots and extended into "dry" soil at varying degrees of possible relative humidity, as follows: Upper left, Series A, approximately 50 per cent.; lower left, Series B, 82 per cent.; upper right, Series C, 99 per cent.; lower right, Series D, 100 per cent. Thirty days after germination.

After 30 days all the remaining 13 pots were removed and photographed. In every case, regardless of the conditions of relative humidity, many roots had extended into the "dry" soil, as is shown in figure 2. The surfaces of the roots were moist with exudate and in most cases had a thin sheath of adhering soil grains. Such a sheath was frequently observed by MAGISTAD and BREZEALE (6). That the soil about the roots was "dry" is evidenced by the fact that no brushing or other treatment was needed to free the roots of soil as they are shown in the photographs. Throughout the experimental period the resistances of the Bouyoucos blocks in the "dry" soil about these 13 pots were of the order of 250,000 ohms. Bouyoucos and MICK (1) state that the resistance at the permanent wilting percentage is around 100,000

ohms. Two samples (of 200-300 gm. each) of "dry" soil were taken from about each pot for moisture determination. The maximum, minimum, and mean percentage values, respectively, were as follows: Series A, 1.33, 0.70, and 1.00; Series B, 1.71, 1.11, and 1.29; Series C, 1.58, 1.16, and 1.27; and Series D, 1.25, 0.36, and 0.86.

All these moisture values are far below the permanent wilting percentage and all indicate a build-up of moisture in the "dry" soil. Since no water or solution was present in the cans of Series A, and the build-up of moisture in the soil of that series was practically as great as in either of the other series, the build-up in the "dry" soil must be attributed to the presence of roots rather than to absorption of water by the soil from the atmosphere surrounding it.

The tar-paraffin mixture was soft and plastic at the temperature of the experiment. The roots were continually growing and expanding, which tended to maintain extremely intimate contact between the root surface and the periphery of the hole in the pot wall at the point of penetration, preventing the seepage of water, alongside the roots, through the pot wall. Moreover, the surface forces of the "dry" soil, and its attraction for water, as the roots entered it, were much greater than the surface forces of the plant root. In such a case a film of water would not flow along a plant root surface through the "dry" soil, but would be "pulled" from the root surface and absorbed by the first dry soil with which it came in contact. For similar reasons water poured on a column of dry soil does not flow freely downward through any root channels or other openings that may exist, but is absorbed by the dry soil and moves downward as a moisture front. There is no reason to think that the build-up of moisture in the "dry" soil of this experiment was due to the flow of water along the root surface from the moist soil inside to the "dry" soil outside the pots. It must be attributed to the conductance of water through the tissues of the roots.

From the standpoint of the moisture stresses involved it is to be expected that plant roots will give up water and build up the moisture content of soil in which the moisture tension (or negative pressure) is higher than that of the root sap. The flow of water in plant roots is not polar. It is a familiar fact that water moves out of plant tissues and plasmolysis occurs if they are placed in solutions having greater osmotic pressures than the cell sap. MAGISTAD and BREZEALE (6) believed that there is an equilibrium between the plant and the soil, and with some plants at least, water moves either from the soil to the plant or from the plant to the soil, depending upon the nature of the moisture gradient. RUSSELL (7) states that the osmotic pressure of the root sap of most plants is of the order of 7 to 165 atmospheres as the soil moisture nears the wilting coefficient; for many plants it is around 15 to 20 atmospheres. The moisture tension in air-dry soil is around 1000 atmospheres (7); in soil at the permanent wilting percentage it is approximately 15 atmospheres. The fact that the soil moisture tension and the osmotic pressure of the root sap are both approximately 15

atmospheres in soil at the permanent wilting percentage may explain why plant root systems can build up the moisture content of dry soil to the wilting percentage but not above it, as BREZEALE (2) observed.

At the end of the experiment the aerial portion of the corn plants was tested with a Geiger counter for the presence of radiophosphorus. None was detected. This, however, cannot be taken as proof of the inability of roots to absorb nutrients from dry soil when moisture is available to them elsewhere. BREZEALE (2) showed that wheat seedlings were able to absorb potassium from soil initially at the permanent wilting percentage when water was available elsewhere in the root system. Had the corn been permitted to grow for a longer period, absorption of phosphate from the "dry" soil might have occurred. Relatively little of the "dry" soil was in contact with the roots. There is no way of estimating with any accuracy the amount of radiophosphorus in sufficiently close contact with the roots for absorption to take place under favorable circumstances. The presence of a probably ample supply of phosphorus in the moist greenhouse potting soil mixture in which the plants grow is a further factor which may have inhibited absorption of radiophosphorus from the dry soil. The results of this test are not conclusive as regards nutrient absorption from initially dry soil, but it is felt that a technique similar to the one employed can be used to answer the question. The authors attempted by another technique (5) to study the absorption of nutrients from soil in the wilting range.

#### Summary and conclusions

The extension of plant roots into dry soil was investigated. Corn was grown for 30 days in tar-paraffin pots filled with moist soil and surrounded by air-dry soil containing radiophosphorus. Bouyoucos blocks were installed in the "dry" soil for the periodic determination of its moisture condition. Variable conditions of relative humidity were maintained about the "dry" soil.

In all cases the corn roots penetrated the walls of the pot and extended into the "dry" soil. The moisture content of the "dry" soil increased, but values as high as the permanent wilting percentage were not obtained.

The results of the experiment indicate that the roots of corn are able to elongate into dry soil and to build up the moisture content of that soil. No evidence was obtained for the absorption of nutrients from dry soil by plants.

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# THE EFFECTS OF LIGHT, GRAVITY, AND CENTRIFUGAL FORCE UPON THE TROPIC RESPONSES OF BUCKWHEAT SEEDLINGS<sup>1</sup>

H. L. CHANCE AND JAMES M. SMITH

## Introduction

The tropistic responses of plants have long interested botanists. They are so evident that one of the earliest botanists observed they could "scarcely have escaped the notice of the most inattentive observer of vegetation."

WENT and THIMANN (3) state that as early as 1758 Du Hamel proved the radicle of an inverted seed would reverse and turn downward while the plumule turned upward. He also conceived the idea of two saps, one moving upward and the other downward, controlling the growth. KNIGHT (2) decided that if, as some naturalists thought, the directional growth of plant shoots is caused by gravity, its effect might be suspended by the action of centrifugal force. In his well-known experiments with seeds of the common garden bean fastened on the perimeter of a wheel and rotated, he found that the shoots grew toward the center of the wheel. They even grew past the center, but then reversed, growing toward the center of the wheel again. He also found that the angle of the shoots from the perpendicular increased with an increase in the speed of the rotation of the wheel.

These experiments were made in an attempt to correlate quantitatively the influence of gravity, centrifugal force, and light upon the directional growth of shoots. Gravity is a fixed factor for a definite earth position and is used as a standard of comparison. The centrifugal force was developed by a wheel having a radius of 35 centimeters. The light source was a 100-watt Mazda lamp in 110-115 volt circuit. Since stimuli act through time, the unit taken as a standard was the time required for shoots placed in a horizontal position in the dark to assume a vertical position; *i.e.*, three hours. The temperature for all experiments ranged from 26° to 28° C.

The giant form of the Japanese variety of buckwheat (*Fagopyrum esculentum*, Moench) was chosen since preliminary experiments indicated giant forms to be more sensitive than dwarf forms. The seeds were selected and placed in moist chambers to germinate and seedlings were selected from these.

The plants were grown in soil consisting of two-thirds sand and one-third loam. The soil was placed in ten-ounce paper cups and the germinated seeds planted in a row across each cup. Two 200-watt Mazda lamps were placed eighteen inches above the cups to facilitate straightening of the seedlings as quickly as possible after emerging from the soil. The plants were usually grown and prepared in the same room in which the tropic reactions were studied. When ready for use, the shoots were thinned to

<sup>1</sup> Contribution from the Department of Plant Sciences, University of Oklahoma, no. 84.



three or four in each cup by clipping off near the soil. The remaining shoots were selected for straightness, size, and spacing. Their length was then determined and the shoots were ready for use. After being subjected to treatment for three hours, their curvatures were measured by means of a protractor such as used and illustrated by WENT and THIMANN (3).

In experiments using centrifugal force a plywood platform, thirty-six inches in diameter, was constructed on a steel wheel, formerly part of a wheel chair. A circle, concentric from the center of the wheel, with a radius of 35 centimeters was drawn on the platform. With a known radius the centrifugal force could be computed in terms of gravity according to the formula:

$$C. F. = 0.0000112 W^2 r$$

When C. F. = Centrifugal Force

W = Revolutions per minute

And r = radius in centimeters

Eighteen metal cups, containers for the culture cups, were spaced around and outside the circle in such a position as to allow the top of the culture cups to be flush with the line. The metal cups were fastened on the platform with "L"-shaped iron strips. They were secured to the strips by a bolt passing through a slot about an inch long cut in the strips and cups. This device made it possible to adjust the metal cups to any desired angle. In order to prevent air currents and light from passing from one side across center to the other side, nine partitions of black window shade cloth supported by wire frames were placed on the platform radiating from the center to perimeter, thus dividing the platform into nine equal wedge-shaped compartments. There were two metal cups in each compartment. A sun-ray glass (trade name for ordinary screen wire covered with translucent plastic material) screen encircled the wheel to the height of the partitions. A lid of cardboard covered with black cloth was used in experiments in the dark. The wheel, turned by a one-third-horsepower motor, was not operated directly from the motor, but from an intervening set of pulleys of various sizes depending upon the speed desired.

### Methods and results

The response time was arrived at by placing the plants in a dark room with the shoots in a horizontal position and noting the time required for the shoots to assume a vertical position. The basement lights were turned on and the measurements made immediately. The time required was three hours. This was used as the reaction time for all other experiments.

It was next desirable to determine the least centrifugal force required to influence directional growth. If plants are placed in a vertical position on a wheel revolving in a horizontal plane, gravity influences curvature. To overcome this influence the wheel was revolved in a vertical plane with shoots at right angles to the radius of the wheel. The wheel then served as



a klinostat and gravity was neutralized. The speed required for the wheel to act as a klinostat was not determined but only the speed necessary to produce a centrifugal force sufficient to influence curvature. The speed was found to be between 7 and 8 r.p.m. or 0.019 to 0.025 earth pulls. There was no response at 0.019 earth pulls and at 0.025 earth pulls 33 of the 161 shoots gave no curvatures, while the remainder gave definite responses. The curvatures for the individual shoots varied from  $0^{\circ}$  to  $13^{\circ}$  with an average of  $5.46^{\circ}$ . This figure is based on four separate experiments. The centrifugal force equivalent to 0.025 of an earth pull was taken as the sensitivity point for these shoots. The results compare favorably with those of DARWIN and PERTZ (1) who found that the plants with which they worked responded to a centrifugal force of 0.02 to 0.05 earth pulls.

Though KNIGHT (2) found that the angle of shoots from the perpendicular increased with the speed of the wheel, he made no attempt to measure curvature at known speeds. To make these determinations the wheel was changed to a horizontal position for all other work. The wheel was adjusted while loaded with cups of soil, thus giving it the same load it would have during the experiments, to the speed at which the experiment was to be made. The cups containing the plants were numbered; each shoot was measured and its length recorded when placed on the wheel. At the end of the response time, the angle of curvature was recorded for each shoot.

A third factor to determine was the location of the point of balance between gravity and centrifugal force in the dark. Substituting in the formula  $C. F. = 0.0000112 W^2 r$ , it was found that the wheel must rotate  $50.5 + r.p.m.$  to develop a centrifugal force equivalent to an earth pull. Theoretically, plants placed at any angle and rotated at this speed should give angles which would bring them to a line parallel to the resultant of the two forces or to an angle of  $45^{\circ}$ .

One hundred fifty-seven plants, representing four separate experiments, were placed on the wheel in a horizontal position and rotated at  $50.5 + r.p.m.$  for three hours. The average curvature was found to be  $44.93^{\circ}$  upward (table I).

This was repeated with the plants placed in a vertical position. The shoots gave an average curvature of  $46.06^{\circ}$  downward or  $43.94^{\circ}$  from the horizontal. When 66 plants were placed on the wheel at an angle of  $65^{\circ}$  from the vertical, the average curvature was found to be  $21.01^{\circ}$  upward. This angle, plus the angle of the shoots at the beginning,  $25^{\circ}$  from the horizontal, gives a total of  $46.01^{\circ}$  from the horizontal. Fifty-six plants were placed at an angle of  $35^{\circ}$  from the vertical and rotated as above. The average curvature was  $12.05^{\circ}$  downward. The angle at the beginning,  $35^{\circ}$  from the vertical, plus the angle of curvature, makes a total of  $47.05^{\circ}$  downward or  $42.95^{\circ}$  from the horizontal. Thus for the four positions, *i.e.*, horizontal, vertical,  $65^{\circ}$  from the vertical, and  $35^{\circ}$  from the vertical, the final positions taken by the shoots after treatment were 44.93, 43.94, 46.01 and  $42.95^{\circ}$  upward from the horizontal positions or a variation of slightly more than three degrees.

The curvatures were also determined for both horizontal and vertical positions of shoots subjected to 0.96 earth pulls which were obtained at a speed of 49.5 r.p.m. One hundred ninety-eight plants placed in a horizontal position and rotated for the reaction time gave an average curvature of  $50.70^\circ$  upward. One hundred fifty-eight plants placed in a vertical position were rotated as above and gave an average curvature of  $37.39^\circ$  downward. The response in both cases was in accordance with the assumption that the shoots would give curvatures in the direction of the greater stimulus. This point was further established when the shoots were subjected to a centrifugal force of 1.04 earth pulls. One hundred and one shoots placed in a horizontal position gave an average curvature of  $40.46^\circ$  upward while 157 shoots placed vertically gave an average curvature of  $50.59^\circ$  downward.

TABLE I

SUMMARY OF ALL CURVATURES FROM THE HORIZONTAL POSITION OF PLANT SHOOTS POINTING TOWARD THE CENTER OF THE WHEEL WHEN ROTATED AT 50.5+ R.P.M. (1 EARTH PULL). TEMPERATURE 26-28° C.

LENGTH OF SHOOTS	No. of PLANTS	38°	39°	40°	41°	42°	43°	44°	45°	46°	47°	48°	49°	50°	51°	52°
cm.																
2½	14	.....	1	1	.....	1	.....	3	3	1	1	1	2	.....	.....	.....
3	25	1	.....	.....	2	.....	2	3	7	5	2	.....	1	1	.....	.....
3½	34	.....	1	2	2	1	4	3	4	4	6	3	1	2	1	.....
4	36	.....	2	1	.....	3	1	5	9	4	2	1	5	2	1	.....
4½	19	.....	1	2	.....	2	.....	6	6	7	.....	.....	.....	.....	.....	1
5	16	.....	.....	.....	3	1	2	1	2	.....	2	2	2	.....	1	.....
5½	10	.....	.....	.....	1	.....	.....	2	1	2	.....	.....	2	.....	2	.....
6	3	.....	.....	.....	.....	.....	2	.....	1	.....	.....	.....	.....	.....	.....	.....
Totals	157	1	5	6	8	6	13	17	33	23	13	7	13	5	5	1

Average curvature 44.93 degrees upward.

The preceding experiments were carried out in the absence of light. Next it was desired to add this factor to similar experiments. In order to do so, it was necessary to determine the influence of light as compared to that of gravity. This was done in two ways. First the plants were placed upright on the floor and illuminated unilaterally at various distances from a 100-watt Mazda lamp which was placed in a black box with an open end toward the plants. Cups containing the plants were placed at various distances from the light source and left for the three-hour reaction period and the curvatures determined. The distance from the light source at which the shoots (30 in number) approached an average angle of  $45^\circ$  in the reaction time was 120 centimeters. The individual shoots at this distance varied in curvature from  $43^\circ$  to  $48^\circ$  with an average of  $44.6^\circ$ . In the second method the plants were placed in a horizontal position at various distances above the light source. Again the point of balance was found to be 120 cm. from the light source. At this distance the shoots of six plants gave no curvature while those of twelve plants curved upward and thirteen downward.

In balancing light plus gravity against centrifugal force, it was necessary to use two sets of experiments in order to keep the light source the same distance from the plants. The first experiments were run with the plants in a horizontal position with the light source 120 cm. above the plants. In the second group of experiments the plants were placed upright which necessitated moving the lights upward a corresponding distance.

Four lights instead of one were used, the lamps being equally spaced around the wheel above the plants. A shade was arranged around the lights to restrict the illumination and prevent reflections which would strike the plants from the sides. Cross partitions were used to separate the four lights to prevent the rays from crossing and giving more illumination than was afforded by one light.

When the plants were placed in a horizontal position and the light source as described placed 120 cm. above, the shoots were subjected to one earth pull plus its equivalent in light. Theoretically a centrifugal force of two earth pulls would be required to balance the two forces and was obtained by rotating the wheel at a speed of approximately 72 r.p.m. Four experiments were run at this speed with the plants in a horizontal position. The average curvature of the 229 shoots involved was  $45.25^\circ$  upward. Six experiments were made with the plants in a vertical position. The average curvature for a total of 356 shoots was  $45.71^\circ$  downward. The results substantiate the hypothesis that the stimuli of gravity and light are directly additive in respect to centrifugal force in effect upon the curvatures. This principle was further borne out by checking the curvatures of shoots at approximately 1.9 and 2.1 earth pulls in terms of centrifugal force. Four experiments were made consisting of 276 plants placed in a horizontal position and subjected to a centrifugal force of approximately 1.9 earth pulls. The average curvature of the shoots was  $50.94^\circ$  upward; of the two hundred eighty-nine plants placed vertically and treated as above, the average curvature of the shoots was  $41.16^\circ$  downward. Next 300 plants were placed in a horizontal position and subjected to a centrifugal force equivalent to approximately 2.1 earth pulls. The average curvature of the shoots was  $38.39^\circ$  upward, while 292 plants placed in a vertical position and given similar treatment gave an average curvature of  $52.70^\circ$  downward.

It was found that the tropic influence of gravity plus its equivalent in light could be counteracted by a centrifugal force of two earth pulls. Under this condition the shoots of those plants placed in a horizontal position averaged an angle of  $0.25^\circ$  above while those in a vertical position averaged an angle of  $0.71^\circ$  below that of the balance point. As could be expected, the shoots of the plants subjected to a centrifugal force of 1.9 earth pulls did not come to a position of  $45^\circ$ , but to an angle above  $45^\circ$ , thus giving the greater response to the greater stimuli of gravity and light. The shoots of the plants in a horizontal position averaged an angle  $5.94^\circ$  above the point of balance while for those in a vertical position it was  $3.84^\circ$  above the balance point. When the plants were subjected to a centrifugal force of 2.1 earth

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pulls, the shoots again gave a greater response to the greater stimulus. The shoots placed in a vertical position were brought to position of  $7.70^\circ$  below the balance point while those in the horizontal position were brought to positions averaging  $6.61^\circ$  below the balance point of  $45^\circ$ .

The reaction time of shoots was determined in respect to gravity plus its equivalent in light. The plants were placed in a horizontal position and the light source placed 120 cm. above. The time required for the shoots to change from a horizontal to a vertical position was one hour and fifty minutes whereas it required 3 hours in the absence of light. These data do not indicate that gravity and light are directly additive in respect to time in their combined influence upon curvatures.

The next step was to balance light against centrifugal force but due to mechanical difficulties this experiment was abandoned. However, since gravity and its equivalent in centrifugal force have been found to have comparable effects and gravity plus its equivalent in light have been found to be additive as to centrifugal force, then light should balance centrifugal force in its tropic effect upon buckwheat seedlings. This leads to the assumption that light, gravity, and centrifugal force can be expressed in the same terms of earth pulls in considering their effects on tropic responses of buckwheat shoots.

### Summary

1. The time required for the shoots of buckwheat seedlings placed in a horizontal position, in the dark and at a temperature of  $26-28^\circ$  centigrade, to assume a vertical position was three hours.

2. The least centrifugal force exerted in a vertical plane required to induce tropic response in buckwheat shoots was found to be between 0.019 and 0.025 earth pulls which required about 8 r.p.m. when shoots were 35 cm. from the axis of the wheel. The average curvature at this speed was 7.16 degrees. At 0.019 earth pulls, the shoots gave no response.

3. When a centrifugal force equivalent to one earth pull was exerted upon shoots in a horizontal position the shoots assumed a position parallel to the resultant of the two forces or an angle of  $44.93^\circ$ , as compared to the theoretical of  $45^\circ$ . The shoots of the plants placed in a vertical position and subjected to the same treatment gave an average curvature of  $46.06^\circ$  downward. When the centrifugal force was changed to 0.96 earth pull, the shoots in the horizontal position gave an average curvature of  $50.70^\circ$  upward while those placed in a vertical position gave an average curvature of  $37.39^\circ$  downward. If the centrifugal force was changed to 1.04 earth pulls, the shoots in a horizontal position assumed an average angle of  $40.46^\circ$  upward and those placed in a vertical position  $50.59^\circ$  downward.

4. The distance at which shoots approached an angle of  $45^\circ$  in the reaction time of three hours when unilaterally illuminated was 120 cm. from the light source, when using a 100-watt Mazda lamp in 110-115 volt circuit. The average of the angles at this distance was  $44.6$  degrees.

5. The distance from the light source at which the shoots gave the least

curvature in the reaction time when illuminated from below was 120 centimeters.

6. The influence of one earth pull plus its equivalent in light upon the curvature of shoots was offset or balanced by a centrifugal force of two earth pulls.

7. The reaction time of shoots placed in a horizontal position and illuminated from above with the light source 120 cm. away was one hour and fifty minutes. The difference in the reaction times of shoots in the dark and in the light was one hour and ten minutes.

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## MICROMANIPULATIVE STUDIES ON GELATINIZED STARCH GRANULES

M. J. COX AND M. M. MACMASTERS

(WITH TWO FIGURES)

Gelatinized starch granules are generally considered to be comprised of sacs or outer membranes of starchy material enclosing a more or less fluid colloidal starch dispersion [see reviews by RADLEY (10)] and KERR (6). NÄGELI (9) and MEYER (8) considered the membrane to be preexistent in the native, ungelatinized granule. ALSBERG (1) and BADENHUIZEN (2, 3) on the other hand, concluded that the membrane is formed as an artifact by the swelling and coalescence of the more resistant lamellae. Experimental results reported by HESS and RABINOWITSCH (5) and further discussed by ROBERTS (11) indicate that, however it may be formed, the outer membrane of the gelatinized granule is tough and can be punctured by a needle, releasing a fluid colloidal suspension with which the sac is filled.

During the course of an investigation at this Laboratory of the factors affecting starch paste viscosity, micromanipulative studies were made of the degree to which gelatinized granules of corn, potato, tapioca, and glutinous corn starch could be stretched. Several hundred granules of each kind of starch were observed, each granule being stretched until broken. The main purpose of the study was to determine whether length of paste might be correlated with ability of the individual unbroken granules to stretch. (A long paste has a small yield value and low mobility.) Information was also obtained on the degree to which starch granules could be stretched after pasting at different temperatures and for different lengths of time. Some effects of chemical pretreatment of starch granules upon their subsequent ability to stretch were also noted.

The starches used in these experiments included commercial tapioca starch, laboratory-processed glutinous corn starch, and both commercial and laboratory-processed corn and potato starches. Nitrogen and ash contents of these starches, determined as described by MACMASTERS and HILBERT (7), are given in table I. The laboratory-processed samples of corn starches were prepared by the method described by Cox, MACMASTERS, and HILBERT (4).

### Methods

In order to move and stretch the starch granules, fine glass needles were used in a pair of Fitz micromanipulators which permitted movement in three planes. Gelatinized starch granules were stretched at room temperature and at approximately 40° C. For stretching at room temperature a glass micromanipulator cell of conventional type was used. The gelatinized starch was suspended in a small drop of water on a cover slip and the slip



TABLE I  
DESCRIPTION OF STARCH SAMPLES USED

SAMPLE NUMBER	STARCH	METHOD OF PREPARATION	PERCENTAGE ON DRY BASIS	
			N	ASH
1	Tapioca	Commercial	%	%
2	Glutinous corn	Laboratory 0.1% SO <sub>2</sub> steep	0.01	0.09
3	Corn	Commercial SO <sub>2</sub> "	0.04	0.04
4	Corn	Laboratory H <sub>2</sub> O "	0.05	0.06
5	Potato	Commercial No steep. SO <sub>2</sub> processed	0.03	0.07
6	Potato	Laboratory " " H <sub>2</sub> O "	0.01	0.03
			0.03	0.04

inverted over the opening in the center of the glass cell. A similar micro-manipulator cell heated by water circulated from a constant temperature bath was employed for samples stretched at 40° C.

Individual starch granules were stretched by placing two micro-needles upon the granule and slowly pulling the needles apart. The needles were placed near the center of the granule, then as they were pulled apart, they slipped over the surface of the granule until near the granule periphery. Thus stretch was measured throughout essentially the whole length of the granule. Considerable care was necessary in placing the needles upon a granule so as not to puncture it prior to stretching. When granules were biaxial, as in potato starch, they were pulled so that the stretch would be along the long axis. For the starches which had approximately spherical granules, alignment was necessarily at random. The needles were pulled apart slowly. Rapid or jerky movement of the needles caused the granules to break before the maximum degree of stretch was reached. When the granules were stretched and broken, the granule fragments would snap back around their respective needles. Measurements were made with a microscope equipped with a calibrated ocular micrometer. The percentage stretch of each granule was calculated.

$$\text{Percentage stretch} = \frac{\text{Stretched diam.} - \text{original diam.}}{\text{Original diam.}} \times 100.$$

Percentage of stretch within any one sample varied considerably. Twelve corn starch granules from a sample pasted for 10 minutes in a boiling water bath and stretched as soon as they had cooled to room temperature (25° C.) gave 55 to 222 per cent. stretch, average 127. Another 13 granules, prepared and stretched in an identical manner gave 100 to 207 per cent. stretch, average 150. For 140 corn starch granules, including the 25 already mentioned, the average stretch was 157 per cent. These granules were chosen at random from 11 different samples. These data were analyzed statistically and it was found that the probability is about 99 per cent. that the mean of the sample, 157, is within 10 of the mean of the population of starch granules; *i.e.*, it is practically certain that the mean of the stretch of starch

granules taken from similar starch pasted under similar conditions will fall within the range of 147-167 per cent.

## Results and Discussion

### NATURE OF THE INTERIOR OF STARCH GRANULES

ROBERTS (11), in a résumé of work which he had carried out earlier with HESS (5), reported that with an ultramicroscope Brownian movement could be seen within gelatinized starch granules to which alcohol or other organic solvent had been added. BADENHUIZEN (3) reported that upon pricking an untreated swollen starch granule with a needle the granule did not crumple nor did any material flow out from it. Repetition of the work of these two authors confirmed the results of both. The apparent conflict

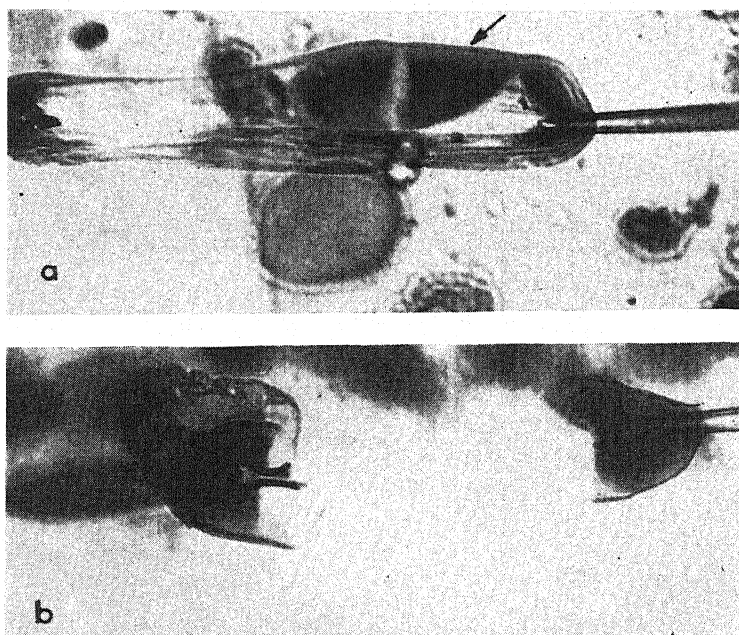


FIG. 1. Gelatinized potato starch granules stained with 1 per cent.  $I_2KI$ . a. Maximum amount of stretch. Note gelatinous center at tip of arrow. b. Granule broken and snapped back around needles.  $\times 135$ .

in their results can be attributed to the use by ROBERTS of an organic solvent which causes precipitation of the colloidal material within the liquid contents of the granule.

Gelatinized potato starch stained with approximately 1 per cent.  $I_2KI$  solution appeared to have a firm gelatinous center (fig. 1a) around which there was a clear transparent margin. When these granules were stretched and broken, the break was clean (fig. 1b).

When 0.01 per cent. solution of  $I_2KI$  was used, the interior of the granule was not precipitated and the break was ragged. It was reported by BADENHUIZEN (3) that the iodine staining hardened the granules and made them

much more difficult to cut than unstained granules. The clear margin which surrounds the dark-stained center of the granule is attributed by BADEN-HUIZEN (3) to the thinness of the granule in this region.

In order to determine whether reorientation of the carbohydrate molecules within the starch granules could be brought about by stretching swollen starch granules, a commercial corn starch (No. 3, table I) was gelatinized in a boiling water bath and stretched between crossed Nicol prisms. The granules were stretched parallel to the axis of the polarizer and no birefringence was observed. The micromanipulator needles were then set approximately  $30^\circ$  from the axis, but still no birefringence was exhibited by the stretched granules. Although it is known that in many instances the addition of  $I_2KI$  solution will bring about a return of birefringence to gelatinized starch granules (unpublished data), when granules were stained with iodine solution and subsequently stretched between crossed Nicol prisms, no birefringence was seen.

#### EFFECT OF TEMPERATURE AT TIME OF STRETCHING

The length to which granules could be stretched at  $25^\circ C.$  was not significantly different from that to which similar granules could be stretched at  $40^\circ C.$  This was true for each starch studied regardless of pasting temperature and time.

#### EFFECT OF PASTING TEMPERATURE

A significant increase was found in the percentage stretch of granules with increasing temperature of pasting for both corn and potato starches. Corn starch granules pasted at  $80^\circ C.$  showed a mean stretch of 106 per cent. compared with 152 per cent. for those pasted in a boiling water bath. In the case of potato starch the difference between samples pasted at  $80^\circ C.$  and  $90^\circ C.$  was highly significant, the respective averages of stretch being 140 and 185 per cent.

#### EFFECT OF PASTING TIME

Results of the statistical analysis of data (table II) collected on commercial corn starch (No. 3, table I) pasted at  $80^\circ C.$  for 10, 30, and 60 minutes and for 72 hours showed that the percentage of stretch in samples

TABLE II

PER CENT. OF STRETCH OF STARCHES SHOWING NUMBER OF ITEMS IN SAMPLE AND THE STANDARD DEVIATION OF SAMPLES PASTED AT  $80^\circ C.$  FOR SPECIFIED LENGTHS OF TIME

STARCH/STATISTICAL MEASURE	TIME OF PASTING			
	10 min.	30 min.	60 min.	72 hours
Corn starch				
Mean stretch (%) .....	94	126	100	84
No. in sample .....	12	12	12	20
Standard dev. ....	23	34	27	45
Potato starch				
Mean stretch (%) .....	.....	135	.....	77
No. in sample .....	.....	50	.....	15
Standard dev. ....	.....	37	.....	18

pasted 30 minutes is significantly greater than that in samples pasted for all other lengths of time used (table III). No difference was shown among samples pasted for 10 minutes, 60 minutes, and 72 hours. From the available data, it is impossible to say at what time between 10 minutes and 1 hour maximum stretch would occur.

Although the data for potato starch (No. 5, table I) are incomplete, granules pasted for 30 minutes at 80° C. were found to stretch considerably more than granules from a similar sample pasted for 72 hours (table II). There was a highly significant difference in the length to which granules pasted for these two time intervals would stretch before breaking.

TABLE III

SIGNIFICANCE OF DIFFERENCE OF STRETCH BETWEEN SAMPLES OF CORN STARCH PASTED FOR VARYING LENGTHS OF TIME AT 80° C.

TIME OF PASTING	TIME OF PASTING			
	10 MINUTES	30 MINUTES	60 MINUTES	72 HOURS
10 minutes .....	—	*	†	†
30 minutes .....	....	—	*	† *
60 minutes .....	....	....	—	†
72 hours .....	....	....	....	—

\* Indicates 5 per cent. level of significance.

† No significant difference.

‡ Approaches 1 per cent. level of significance.

#### RELATIONSHIP OF STRETCH TO STARCH PASTE VISCOSITY

One of the objects of this investigation was to determine whether there is any correlation between paste viscosity and the ability of individual granules to stretch. There is known to be a rough correlation between length of paste, tackiness, and paste viscosity. For example, corn starch which has a short paste has a low paste viscosity and tapioca and potato starch which have long tacky pastes have a high paste viscosity. In concentrated pastes, in which the granules might be in close contact with each other, the ability of individual granules to stretch might contribute materially to the length of the paste. From the data at hand, however, it would appear that there is no correlation between the ability of a starch granule to stretch and the paste viscosity of the starch.

The MacMichael viscosity (at 95° C., 20 r.p.m.) of 3 per cent. suspensions pasted at 95° C. for 10 minutes with stirring at 120 to 160 r.p.m. is 375, 270, 95, and 140 C.P. for glutinous corn, tapioca, corn and potato starches, respectively. Glutinous corn starch or tapioca starch pasted for 10 minutes in a boiling water bath could be stretched to about 3 times or 200 per cent. of the original granule length (fig. 2) as compared with 157 per cent. for corn and 145 per cent. for potato starches.

The stretch of pasted granules of corn starch which had been processed with sulfur dioxide (No. 3, table I) was compared with the stretch of simi-

larly pasted granules of the same kind of starch processed with distilled water (No. 4, table I). The latter had higher viscosity. There was no more difference in stretch between these two starches than there was among granules within either sample. A portion of commercial corn starch (No. 3, table I) was shaken for one hour in N/32 NaOH solution and subsequently washed free of alkali. This treatment increased the paste viscosity of the

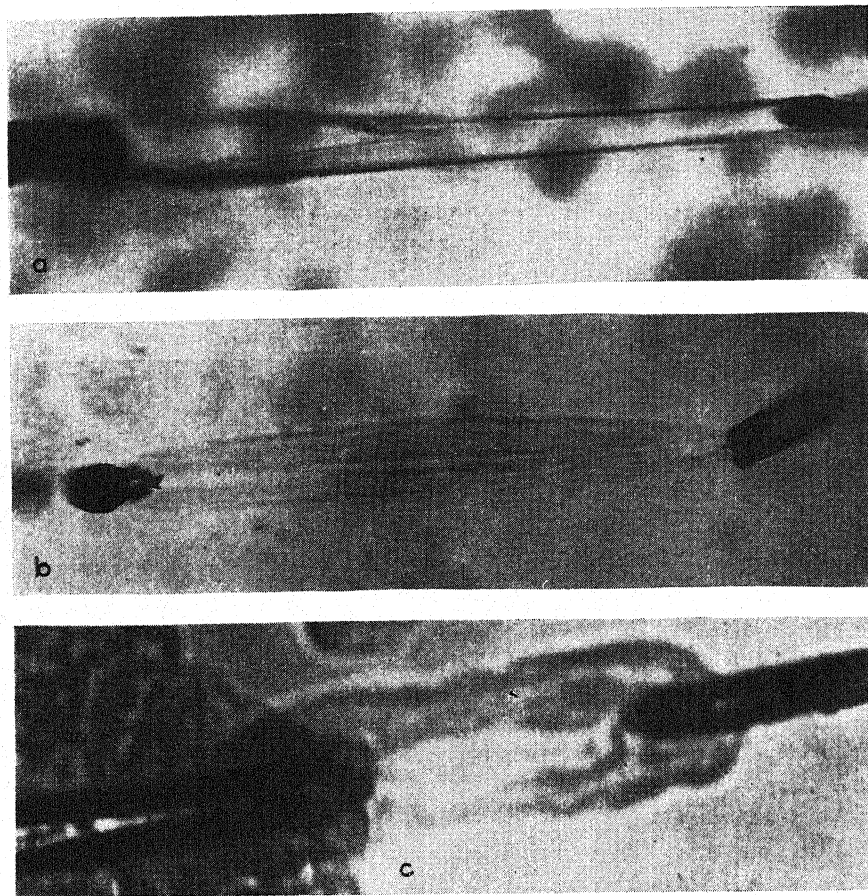


FIG. 2. Starch granules stained with 0.01 per cent.  $I_2KI$  showing maximum amount of stretch. a. Glutinous corn starch. b. Tapioca starch. c. Corn starch.  $\times 300$ .

starch. The degree of stretch of the pasted granules was, however, the same as for the untreated starch. Treatment of corn starch with dilute solution of either acid or alkali did not alter its potentiality for being stretched.

#### COHESIVENESS OF STARCH GRANULES

Sulfur dioxide treatment had a marked effect on potato starch granule properties. Potato starch processed with water (No. 6, table I) was very difficult to handle with a micromanipulator because of the stickiness of the



gelatinized granules and their tendency to adhere to the cover slip. When a sample of this starch was washed with 0.2 per cent.  $\text{SO}_2$  solution, dried at  $40^\circ \text{C}$ . and subsequently gelatinized and stretched, there was no tendency for the granules to adhere to the cover slip. The treatment also lowered the paste viscosity.

Micromanipulative tests were made on 3, 4, and 5 per cent. pastes of commercial corn starch to determine how easily granules could be separated from the main body of the paste. It was very easy to disengage granules from the 3 per cent. paste. Whenever 2 or 3 granules pulled away together they could be readily separated from one another. In the case of the 4 per cent. paste, it was somewhat harder to separate granules from the paste and from each other than in the 3 per cent. sample. When a microneedle was fastened onto a granule near the edge of the 5 per cent. paste and a pull exerted, a large piece of the paste would separate from the main portion of the sample. It was very difficult to disengage individual granules from this gel.

These samples were again examined after having stood overnight at about  $5^\circ \text{C}$ . In every instance it was more difficult to separate the granules than it had been when the freshly prepared material was used. It was also more difficult to engage a granule with the microneedles after the paste had stood overnight, as the granules became hard and rubbery. Reheating the paste to  $100^\circ \text{C}$ . did not facilitate the separation of an individual granule from the paste nor was it easier to fasten a microneedle onto a granule. The changes which had taken place during refrigeration were therefore not reversible.

Similar tests were made upon potato starch pastes. The 3 per cent. paste was very elastic and although the granules would undergo considerable stretch before pulling away from the paste, individual granules could be separated fairly easily. The 4 per cent. paste was very cohesive but individual granules could be pulled away from the main body of the paste. The 4 per cent. and 5 per cent. pastes were almost identical.

### Summary

1. A micromanipulative study was made of starches, including corn, glutinous corn, tapioca, and potato to determine whether length of paste might be correlated with ability of the individual granules to stretch without breaking. The data show that there is no apparent correlation between these two phenomena.

2. Starch granules pasted at  $100^\circ \text{C}$ . can be stretched to a greater extent than similar granules pasted at  $80^\circ \text{C}$ . for the same length of time.

3. When identical corn starch samples were pasted at a given temperature for 10, 30, and 60 minutes, and 72 hours, a significantly greater stretch was exhibited by the samples pasted for 30 minutes than for the others, all of which were essentially alike in ability to stretch.

4. Pretreatment of starch granules with 0.2 per cent.  $\text{SO}_2$  or  $\text{N}/32 \text{ NaOH}$  did not cause any appreciable change in the percentage of stretch of the



granules. These treatments respectively decreased and increased the paste viscosity.

5. Stretching the gelatinized granules did not bring about realignment of the molecules sufficient to cause a return of birefringence.

6. Individual granules could be pulled away from the main body of a 3 per cent. corn starch paste with ease. Potato starch granules would undergo more stretch than corn starch granules before separating but they, too, came away with ease. It was difficult, however, to separate single granules from a 4 or 5 per cent. corn or potato starch gel.

The authors are indebted to CAROL M. JAEGER for statistical analyses and to members of the Analytical and Physical Chemical Division for analytical data.

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# THE EFFECT OF LIGHT ON THE ELECTRICAL POLARITY AND THE RATE OF ELONGATION OF THE, AVENA COLEOPTILE<sup>1</sup>

A. R. SCHRANK

(WITH THREE FIGURES)

## Introduction

Illumination of plants causes their electrical polarity and growth rate to change. These facts were observed years ago, but the relationship, if any, between these two changes has not been established. The experiments to be presented are concerned with this relationship.

It appears that HAAKE (9) was the first investigator to observe alterations in the electrical polarities of plants caused by changes in illumination. WALLER (16), who cites the literature to 1925, presented evidence for associating the electrical changes of green leaves with the activity of chlorophyll. The etiolated seedlings used by him gave little if any electrical response when illuminated. Since 1925, GLASS (8) and BROWN (5) have shown that the electrical polarity of *Elodea* leaves and internodes of *Chara vulgaris* manifest notable changes when their illumination is varied. SHEARD (12) exposed leaves of the sunflower and Poinsettia to both ultra-violet and infra-red light and obtained potential changes as high as 0.3 volt. Effects of light on the bioelectric potentials of *Halicystis* and *Valonia* have also been noted (2, 10, 11). It happens, however, that the effect of radiant energy on the bioelectrical potentials of etiolated plants has rarely been observed. CLARK (6) uniformly illuminated 30- to 40-mm. etiolated *Avena* seedlings with 600,000 meter-candle-seconds of incandescent light and recorded changes in the longitudinal electrical polarity. His data show a diphasic electrical variation in which the polarity first decreases, then increases before returning to the original value.

Numerous experiments have been performed to show the influence of illumination on the growth of the *Avena* coleoptile. One aspect of this problem deals with the *inhibition* of growth (3). VAN DILLEWIJN (7) subjected the *Avena* coleoptile to uniform illumination and noted effects on the growth rates by both continued and short-period irradiation. After a latent period, he observed a period of depressed growth showing two types of responses. In the first and short response, the maximum growth-rate depression was reached after 30 minutes. The second and longer reaction reached the maximum growth-rate depression after 90 minutes. It has been demonstrated that the second depression can be detected only when the apex of the plant is illuminated. [WENT (17): tip response.] The short period of depressed growth rate can be observed when either the basal portion or the entire coleoptile is illuminated. (WENT: base response.) The present ex-

<sup>1</sup> Supported by the University of Texas Research Institute.

periments are concerned with the first short period of growth rate depression and the corresponding electrical polarity changes.

### Materials and methods

Coleoptiles of *Avena sativa*, Victory strain (C.I. 2020, obtained from the U. S. Department of Agriculture) were used in all experiments. The procedure for sprouting and growing the seedlings has previously been described (14). Sheaths 30 mm. in length were isolated from 35-mm., actively growing, straight coleoptiles and placed in a glass holder. This holder is shown in the inset of figure 2. The sheath in the holder was always placed in the apparatus (13) with the electrical contacts in position and allowed to remain undisturbed for 2 hours before beginning the experiment. One contact was placed at the apex and the second 15 millimeters basal to the first.

A 100-watt, frosted General Electric mazda lamp was used as the light source. This lamp was housed in a cylindrical reflector 18 cm. long and 11 cm. in diameter. The reflector was so placed that the open end of the cylinder was 40 cm. directly above the apex of the coleoptile. Heat from the lamp was absorbed by a layer of water 2 cm. deep located 15 cm. below the lower rim of the reflector. This arrangement gave a light intensity of 90 foot-candles at the position of the coleoptile. The light intensity measurements were made with a Model 603 Weston Illumination Meter.

The entire length of the sheath was illuminated by placing an inverted (larger base upward) truncated cone underneath it. This cone was prepared by cutting the stem from a 50-mm. glass filtering funnel and silvering it by the Brashear process. The position of the holder was always adjusted to place the basal end of the vertically oriented coleoptile in the center of the larger and upward base of the cone. The light intensity was measured at 5 mm. intervals along the longitudinal axis of the coleoptile position by a single junction thermocouple connected to a galvanometer. This thermocouple with its blackened 2 × 4 mm. receiver, all enclosed in a glass bulb, was held in position and moved by a micromanipulator. The galvanometer readings show the light to be 6 per cent. more intense at the apex than at the base of the sheath.

All experiments were performed at a temperature of 23 ( $\pm 1.5$ ) degrees centigrade in a dark room under a neon light constructed out of ruby glass tubing. The electrical measurements were made with a DUBRIDGE (4) vacuum tube voltmeter using isoelectric ( $\pm 0.5$  mv.) zinc-zinc sulphate electrodes. Glass rings were used as the contacts with Shive's solution in tap water as the contact medium. Elongation was recorded with an ocular micrometer.

### Experimental data

#### EFFECT OF A 20-MINUTE ILLUMINATION PERIOD ON THE ISOLATED SHEATH

All experiments were started at the end of the two-hour rest periods.

Longitudinal E.M.F.'s, temperature, and elongation readings were started and taken at 5-minute intervals during the remainder of each experiment. Thirty minutes after the beginning of each experiment the light above the coleoptile was turned on and left on for an arbitrary period of 20 minutes. This period of illumination is indicated in figure 1A by the heavy bar below the base line of the E.M.F. curves.

Curves I and II in figure 1A show the changes in the longitudinal electrical polarity of the apical 15 mm. of an isolated coleoptile when illuminated for 20 minutes. These two curves are from duplicate experiments. They are both included because they represent the two typical types of longi-

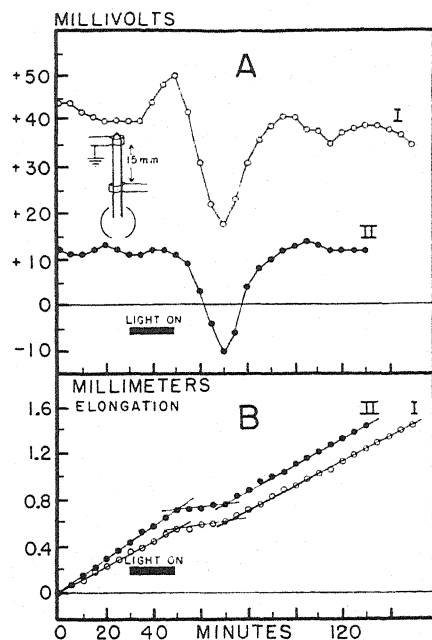


FIG. 1. Effects of a 20-minute illumination period on 30-millimeter, isolated coleoptile sheaths. A. Changes in the longitudinal electrical polarities of the apical 15 millimeters of two sheaths. B. Elongation rates of the same coleoptiles.

tudinal electrical polarity changes in the 15 experiments that were performed. These two curves differ in two respects. First, curve II crosses and goes beneath the 0 millivolt line. This means that the electrical polarity of this coleoptile is inverted for a short period of time. At the present time, no significance can be attached to this inversion. Secondly, there is a small increase in the electrical polarity as shown in curve I while the light was still on. This was observed in about half of the experiments. The total polarity decrease shown by curve I is about the same as shown by curve II. Both E.M.F. curves return to their original value in 60 minutes after the light was turned on.

The elongation curves shown in figure 1B correspond to the E.M.F. curves of figure 1A. The original rates of elongation in 15 experiments

varied from  $8 \mu/\text{min.}$  to  $11 \mu/\text{min.}$ , while the original electrical polarities of these same sheaths varied from 10 mv. in some to 40 mv. in others. Both of the elongation curves show a temporary decrease in the rate of elongation beginning 20 minutes after the light was turned on. All of the 15 experiments that were performed showed this phenomenon without exception. Furthermore, it will be noted that the period of the decreased elongation rate corresponds exactly to the period of decreasing polarity as shown by the curves in figure 1A. Every experiment that was performed duplicated this relationship. After the period of decreased elongation rate, an increased rate was always resumed. (See curves I and II in figure 1B.)

The temperature throughout each experiment was kept constant to within  $\pm 0.25^\circ \text{C.}$

These experiments raise the important question of whether or not the resumption of the original electrical polarity and rate of elongation was due to the light's being turned off after 20 minutes of illumination. The data which follow answer this question in the negative.

#### EFFECT OF A 110-MINUTE ILLUMINATION PERIOD ON THE ISOLATED SHEATH

The procedure used in this series of experiments was identical with the previous one except that the light was left on for a duration of 110 minutes

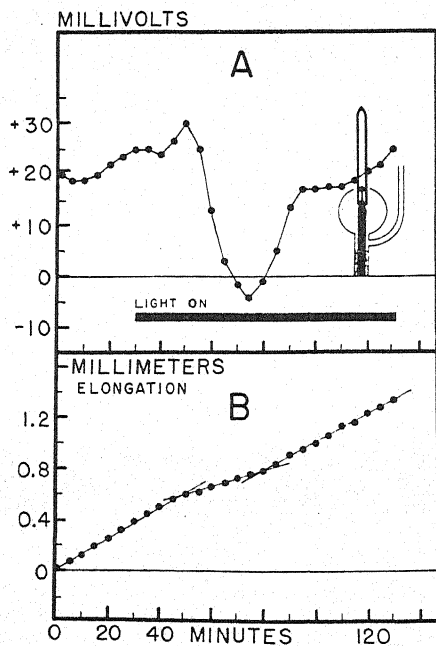


FIG. 2. Effect of continuous illumination on a 30-millimeter, isolated coleoptile sheath. A. Changes in the longitudinal electrical polarities of the apical 15 millimeters of one sheath. B. Elongation rates of the same coleoptile.

instead of 20. Results of a typical experiment on the isolated sheath are shown in figure 2.

The E.M.F. curve shown in figure 2A is similar to the E.M.F. curves in figure 1A in all respects. The electrical polarity starts to decrease 20 minutes after the light is turned on, decreases for 25 minutes, and returns to the original polarity in 60 minutes. The elongation curve in figure 2B shows, as in previous experiments, a period of decreased rate of elongation which corresponds to the period of decreasing electrical polarity. (Compare figures 1B and 2B.) Duplicate experiments using intact 30-mm. plants corroborate these data in all aspects. These curves prove that the return to the original electrical polarity and the resumption of the normal growth rate are not dependent on turning the light off, and they further verify the fact that the period of decreased growth rate corresponds exactly to the period of decreasing electrical polarity.

#### DEPRESSION OF ELONGATION IN APICAL AND BASAL HALVES OF THE COLEOPTILE

In these experiments intact 30-mm. ( $\pm 1$  mm.) coleoptiles were used because they elongate more rapidly than isolated sheaths. An India ink

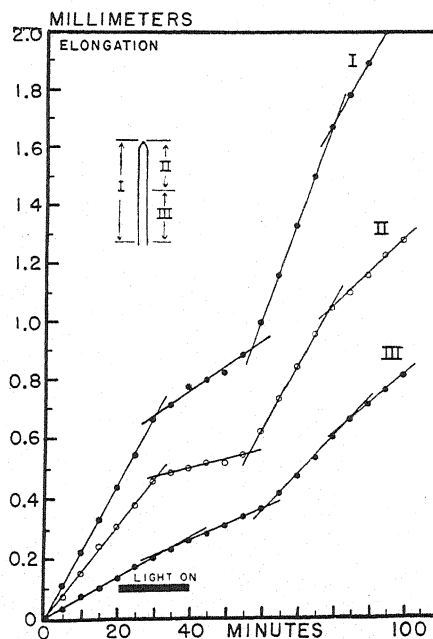


FIG. 3. Elongation rates of intact 30-millimeter *Avena* coleoptiles. I. Elongation of the complete plant. II. Elongation of the apical half. Obtained by subtracting points on curve III from corresponding points on curve I. III. Elongation of the basal half of the coleoptile.

spot was placed half way between the apex and base after which the plant was placed in the holder and allowed to remain undisturbed for 30 minutes. Each experiment was then started by recording the position of the apex and the ink spot by the use of ocular micrometers in horizontal microscopes.



These readings were repeated at 5-minute intervals and continued for 100 minutes. Twenty minutes after the beginning of the experiment the light was turned on and left on for an additional 20 minutes. Figure 3 shows the results of one of these experiments. Curve I represents the elongation of the complete coleoptile and was obtained by recording the position of the apex. Curve III, which shows the growth rate of the basal half, was plotted by following the position of the India ink spot. The growth rate of the apical half of the coleoptile is shown by curve II which was obtained by subtracting the points on curve III from the corresponding points on curve I.

The curves in figure 3 show that incandescent light of 90 foot-candles for 20 minutes causes a temporary decrease of the growth rate of both the apical and basal halves of the *Avena* coleoptile. Comparison of curves II and III indicates that the growth rate in the apical half is more effectively depressed than it is in the basal half. A duplicate series of 10 experiments using the isolated coleoptile sheaths verified these facts. Further inspection of the curves shows that the rate of elongation which is resumed after inhibition is greater in every curve than the original rate. This fact was observed in all experiments. Sometimes this increased growth rate results in a net increase in growth during the period of the experiment as shown by curve III. Why this should occur in some instances and not in others is

TABLE I

ELONGATION RATES EXPRESSED AS SLOPES OF LINES OF 8 INTACT *AVENA* COLEOPTILES, SHOWING ORIGINAL GROWTH RATES, DECREASED GROWTH RATES CAUSED BY ILLUMINATION, AND THE DIFFERENCE IN THESE TWO RATES.  
A SLOPE OF 1 IS EQUAL TO AN ELONGATION RATE  
OF 10 MICRONS PER MINUTE

EXP. NUMBER	A			B			C		
	COMPLETE COLEOPTILE*			BASAL HALF†			APICAL HALF‡		
	ORIGINAL RATE	DECREASED RATE	DIFFERENCE	ORIGINAL RATE	DECREASED RATE	DIFFERENCE	ORIGINAL RATE	DECREASED RATE	DIFFERENCE
1	2.20	0.80	1.40	0.68	0.53	0.15	1.52	0.27	1.25
2	1.60	0.87	0.73	0.48	0.33	0.15	1.12	0.54	0.58
3	1.82	0.93	0.89	0.50	0.33	0.17	1.32	0.60	0.72
4	2.20	0.93	1.27	0.40	0.33	0.07	1.80	0.60	1.20
5	1.54	0.87	0.67	0.34	0.27	0.07	1.20	0.60	0.60
6	1.90	0.60	1.30	0.30	0.13	0.17	1.60	0.47	1.13
7	2.18	1.07	1.11	0.58	0.33	0.25	1.60	0.74	0.86
8	1.50	0.73	0.77	0.46	0.27	0.19	1.04	0.46	0.58
Av.	1.87	0.850	1.01	0.467	0.315	0.153	1.40	0.534	0.866

\* Slopes obtained graphically from plotted lines.

† Same coleoptiles; slopes obtained graphically.

‡ Same coleoptiles; slopes obtained by subtracting slopes in B from slopes in A.

not clear. It will be noted that the period of decreased growth rate shown in figure 3 begins 10 minutes after the light was turned on. In all the other experiments 20 minutes elapsed after turning on the light before the decreased rate became apparent. The data in figure 3 were selected because

they represent all the experiments in this series in all other respects. Examination of the averages of the 8 duplicate experiments performed in this series reveals two other facts. The average duration of the period of decreased growth rate of the entire coleoptile and of the apical half is 19.5 minutes (varying from 16 to 26 minutes). In the basal half the period of inhibition is an average of 7.6 minutes longer. This longer period of inhibition is evident in curve III of figure 3.

The data in table I were taken from the 8 experiments in this group. All of the numbers represent slopes corresponding to the curves of figure 3. A slope of 1 is equal to an elongation rate of 10 microns per minute. Section A of the table comes from growth rates of the entire coleoptile corresponding to curve I. Similarly, section B was obtained from growth rate of the basal halves of the same plants shown in section A. Section C was obtained by subtracting the slopes in section B from the slopes in section A. These slopes correspond to curve II in figure 3. The slopes in the first column of each section represent the original elongation rates while the slopes in the second columns represent the decreased growth rates. The change in growth rates is shown in column three of each section. This column was obtained by subtracting the slopes representing the decreased rates from slopes representing the original rates. This table gives an indication of the uniformity of the experimental results, and the comparison of the average change in elongation rates show that the apical half of the coleoptile is responsible for about 85 per cent. of the growth inhibition.

### Discussion

The changes in the longitudinal electrical polarity of the apical 15 millimeters of the isolated *Avena* coleoptile sheath shown by the data presented are quite similar to the changes recorded for intact plants by CLARK (6). Following the period of decreased polarity, Clark's curves show a period of increased polarity which was not manifested by the isolated sheaths. The data indicate that the isolated coleoptile (with the primary leaves removed) is capable of absorbing radiant energy and responding electrically. Minute quantities of carotenoid pigments, which might well be responsible for the photoreception, have been isolated from the *Avena* coleoptile (15).

The reversible depression of the growth rate of the isolated sheath as shown by the curves in figures 1B and 2B is in agreement with the observations of VAN DILLEWIJN (7). Since the cells in coleoptiles of the lengths that were used are no longer dividing (1), the depressed growth rate must be due to a *decreased rate of cellular elongation*. Whether this decreased rate of cellular elongation is caused by inactivation of the growth hormones, a decreased rate of auxin production, hindrance of hormone transport, or by a change in the reactivity of the cells to the hormones present remains to be demonstrated. The growth responses of the intact coleoptile differ from those of the isolated sheath in two aspects. The intact plant always manifests a period of increased growth rate (faster than the original growth

rate) following the period of decreased growth rate. (Compare figure 3 with the curves in figures 1B and 2B.) The growth rate of the intact plant is always somewhat faster than that of the isolated sheath.

Evidence that is available at the present time indicates that the changes in bioelectric and growth phenomena in the *Avena* coleoptile are related. (1) Electrical and elongation rates change simultaneously. In the dozens of experiments that were performed on both isolated sheaths and intact plants, the period of decreasing electrical polarity is always accompanied by a depressed growth rate. (Figures 1 and 2.) (2) Isolated coleoptiles do not show a period of increased elongation following the period of decreased elongation. Neither do the electrical polarities show a period of increased polarity following the period of decreased polarity. The intact plants show both of these phenomena. (See figure 3 and CLARK (6).) (3) Turning off the light affects neither the electrical polarity nor the growth rate. (Compare figures 1 and 2.) (4) Data from table I show that the apical half of the coleoptile is responsible for about 85 per cent. of the growth rate depression. CLARK'S (6) curves demonstrate that the light-induced electrical polarity changes are practically limited to the apical half of the plant. It is possible that these changes in electrical polarity and growth rates, which are caused by illumination, could be parallel but independent effects. The facts just presented make this seem unlikely. What the linkage is between the decreased growth rate and the decrease in electrical polarity remains to be determined.

#### Summary

1. The apical half of a 30-mm. isolated *Avena* coleoptile sheath responds to uniform illumination by changing its electrical polarity. After a delay of 20 minutes the polarity decreases by 20 to 25 mv. and then returns to the original value.
2. The period of decreasing electrical polarity is always accompanied by a decreased rate of elongation.
3. Continuous and short-period illumination result in similar electrical polarity and growth rate changes.
4. In the intact coleoptile the period of decreased growth rate, which averages 19.6 minutes in length, is always followed by an elongation rate greater than the original.
5. The apical half of the coleoptile is responsible for about 85 per cent. of the growth rate depression.
6. Evidence indicates that the changes in bioelectric phenomena and the changes in rates of elongation are linked.

The technical assistance of MISS GLENDA OGLESBY during this investigation is acknowledged with pleasure.

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# SOME PHYSICAL AND CHEMICAL CHANGES IN THE COMPOSITION OF PECAN NUTS DURING KERNEL FILLING<sup>1</sup>

HARALD E. HAMMAR AND JAMES H. HUNTER

(WITH NINE FIGURES)

## Introduction

Considerable work has been done on the anatomy of the pecan nut and its physiological and morphological development (4, 5, 8, 15, 21, 22, 23). The most complete systematic investigation relative to the development of the fruit of the pecan from an early pre-filling stage to maturity, including changes in the oil, protein, and carbohydrate formation was reported by THOR and SMITH (19, 20) who also gave a comprehensive review of the literature relating to studies on the physiological development of the pecan nut, together with the oil, protein, and carbohydrate formation in the fruit and seeds of other plants. CRANE and HARDY (3) have reported on the effect of cultural and fertilizer treatment on the filling and chemical composition of the pecan, and concluded that the best filled nuts have the highest percentage of oil.

A number of investigators have reported on the analyses of the kernels of mature nuts (3, 5, 12, 17, 19, 22). SHUHART (14) stressed the important function of the pecan hull in the translocation of materials stored in the kernels. SMITH and LOUSTALOT (16, 17) studied the effect of the date of harvest and of curing on the composition of the pecan kernel, shuck, and shell and reported the oil, protein, and carbohydrate changes that took place. FINCH (4) stated that the rapid movement of food materials into the nuts in late summer arose chiefly from those stored elsewhere earlier in the summer.

Very little work has been reported on the mineral content of pecan nuts, especially in regard to the mineral changes taking place during kernel development. HUNTER and LEWIS (7) presented data indicating that the minerals from fertilizers applied at different times during the growing season were taken up and influenced the physiological processes of the tree, as evidenced by differences found in the degree of filling of the nuts at maturity. These observations led them to investigate further the chemical changes occurring in the mineral constituents of the nuts and their supporting shoots at four stages of development. They found (11) that at the period in which the kernel developed and filled, substantial quantities of nitrogen, potassium, magnesium, and phosphorus accumulated in the nut, while the supporting shoots lost significant quantities of all these elements.

Recent studies by the authors (6) showed that a surprisingly large amount of potassium accumulated in the shucks and that there were other

<sup>1</sup> This paper was presented before the Georgia Section of the American Chemical Society held in Atlanta, September 28, 1945.

important changes in the nuts during development. These results stimulated further study of the nitrogen and the mineral constituents of the various parts of the nut during kernel development to determine the part each played, and their effect on the quality of the mature nut.

In a pecan orchard near Albany, Georgia, thirty nuts were picked from clusters of four nuts from each of three trees of the Moore variety at weekly intervals from August 25, 1944, when the kernels were in an early filling (late jell) stage, to full maturity on October 27, 1944. The same trees were used for each sampling, and duplicate determinations were made on the combined sample of thirty nuts from each tree for each of the nine sampling dates.

The shucks were removed, and the nuts were allowed to dry several days in a low temperature oven, after which they were weighed. The shuck samples were dried in an oven at 60° C. and the oven-dry weight was determined. The shucks from the nuts taken on the first three sampling dates had to be removed by cutting and scraping the material away from the shell with a sharp knife, while those from later sampling dates had separated themselves from the nuts. After drying, the nuts were cracked, the kernels were separated from the shells, and the air-dry weights of each were determined.

In preparing the kernel sample for analysis, it was ground in a home-made slicer to pass a 10-mesh sieve and moisture was determined by drying in a vacuum oven at 60° C. for 15 hours at a reduced pressure of 25 inches of mercury. Oil was determined on the air-dry sample using the sulphuric-acid digestion method of LEWIS (10). The shuck and shell samples were ground in a Wiley mill to pass a 60-mesh sieve. One-gram samples of oven-dry kernels and shucks were used for total nitrogen (and protein) determinations, and 5 grams for the determination of ash from which phosphorus, potassium, calcium, and magnesium were determined. Two-gram samples of the oven-dry shell were used for nitrogen determinations, and 10 grams of the material were used for ash and the mineral constituents.

The samples were ashed first under low heat, and finally for 2 hours in an electric muffle at 550° C. Official methods of the A.O.A.C. (1) were used for all the analyses except for potassium, in which the sodium-cobaltinitrite method of BROWN, ROBINSON, and BROWNING (2) was used. Duplicate determinations were made on each sample. Protein in the kernel was calculated by multiplying the total nitrogen by 5.3 (9). Sugars and acid-hydrolyzable polysaccharides were not determined, as sufficient data on these constituents had been determined and discussed in detail by previous investigators (5, 17, 19, 20).

### Results

The data for the mean chemical composition calculated on the percentage of oven-dry weights for the kernel, shuck, and shell materials, and on each of the nine sampling dates, are given in table I. The same data, but calculated on a unit basis of milligrams of constituents per nut, are given in table II.



The data are expressed on the percentage and unit basis because of the striking differences in the curves representing the data obtained for the kernel, and the similarity in the curves representing the data on the shucks and shells of the nuts during development. The data expressed on the unit basis probably show the changes taking place more clearly than when they are expressed on the percentage basis.

TABLE I

MEAN CHEMICAL COMPOSITION OF PECAN NUTS  
PERCENTAGE BASIS

KERNELS								
DATE	NITRO- GEN	PHOS- PHORUS	POTAS- SIUM	CAL- CIUM	MAG- NESIUM	ASH	OIL	PRO- TEIN
1944	%	%	%	%	%	%	%	%
8/25	2.00	0.379	1.228	0.139	0.180	4.56	51.5	10.62
9/1	1.89	0.340	0.686	0.114	0.150	2.81	66.8	10.02
9/8	1.96	0.335	0.441	0.089	0.132	2.48	70.8	10.38
9/15	2.03	0.342	0.405	0.078	0.118	2.50	72.3	10.78
9/22	1.99	0.328	0.399	0.077	0.112	2.19	73.8	10.53
9/29	2.00	0.335	0.428	0.072	0.128	2.41	73.0	10.60
10/6	2.01	0.334	0.427	0.069	0.128	3.15	72.3	10.65
10/13	2.02	0.369	0.448	0.078	0.104	2.65	72.5	10.70
10/27	1.81	0.360	0.394	0.085	0.097	2.47	74.4	9.57
SHUCKS								
8/25	1.35	0.239	2.925	0.790	0.279	9.04		
9/1	1.25	0.219	3.303	0.825	0.293	10.45		
9/8	1.16	0.221	4.377	0.703	0.287	12.45		
9/15	1.09	0.215	4.908	0.637	0.306	13.38		
9/22	1.04	0.206	4.986	0.663	0.291	12.30		
9/29	1.06	0.207	5.075	0.700	0.289	12.61		
10/6	0.99	0.211	4.955	0.795	0.305	13.07		
10/13	1.04	0.202	4.706	0.633	0.294	12.39		
10/27	0.97	0.180	4.022	0.961	0.332	11.87		
SHELLS								
8/25	0.290	0.029	0.163	0.560	0.077	2.26		
9/1	0.282	0.022	0.172	0.559	0.038	2.00		
9/8	0.292	0.020	0.172	0.585	0.012	2.00		
9/15	0.334	0.023	0.170	0.550	0.045	2.06		
9/22	0.318	0.022	0.207	0.541	0.039	2.20		
9/29	0.323	0.020	0.221	0.532	0.031	2.48		
10/6	0.315	0.029	0.211	0.603	0.052	2.26		
10/13	0.320	0.032	0.198	0.540	0.065	2.03		
10/27	0.294	0.026	0.193	0.592	0.040	2.24		

It should be noted that the first sampling date on August 25 did not represent the initial stage of development of the kernel, since over 22 per cent. of the dry weight had been formed prior to the first sampling period, but it does represent the early filling stage.

#### CHEMICAL CHANGES IN THE KERNEL

The oil, protein, dry weight, and ash of the kernel are graphically shown in figure 1 to increase very rapidly during the first three weekly sampling

periods. Similar changes have been shown by other investigators (11, 19, 20). Approximately 96 per cent. of the protein, 82 per cent. of the oil, 85 per cent. of the dry weight, and 84 per cent. of the ash of the kernel at maturity date were formed prior to the fourth sampling date. An indication of the rapid change that takes place in the kernels is that the data show 63 per cent. of the total dry weight, 64 per cent. of the total oil, 43 per cent.

TABLE II  
MEAN CHEMICAL COMPOSITION OF PECAN NUTS  
UNIT BASIS

KERNELS								
DATE	WT. PER NUT	NITRO-GEN	PHOS-PHORUS	POTAS-SIUM	CALCIUM	MAG-NESIUM	ASH	OIL
1944	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
8/25	0.556	11.23	2.11	6.69	0.80	0.95	24.04	350.2
9/1	1.151	21.80	3.87	7.80	1.35	1.72	33.80	772.2
9/8	1.667	32.27	5.50	7.13	1.51	2.24	40.31	1184.5
9/15	2.110	42.96	7.17	8.57	1.70	2.46	49.93	1526.8
9/22	2.370	44.78	7.48	9.13	1.80	2.46	46.22	1703.6
9/29	2.384	47.11	8.05	10.20	1.74	3.09	62.63	1741.5
10/6	2.183	42.70	7.20	9.27	1.59	2.80	70.00	1577.9
10/13	2.296	45.67	8.37	10.07	1.93	2.66	61.05	1665.1
10/27	2.482	44.47	8.84	9.82	2.09	2.42	59.55	1845.2
SHUCKS								
8/25	1.727	23.30	4.13	50.51	13.64	4.82	156.12	
9/1	1.723	21.60	3.77	56.91	14.21	5.05	180.05	
9/8	1.710	19.84	3.78	74.85	12.02	4.91	212.90	
9/15	1.743	18.95	3.75	85.55	11.10	5.33	233.32	
9/22	1.743	18.21	3.59	86.91	11.56	5.07	214.39	
9/29	1.793	19.04	3.71	90.99	12.55	5.18	226.10	
10/6	1.723	17.13	3.64	85.37	13.70	5.26	225.20	
10/13	1.753	18.16	3.61	82.50	11.10	5.15	217.20	
10/27	1.876	18.22	3.38	75.45	18.02	6.23	222.68	
SHELLS								
8/25	2.324	6.74	0.67	3.79	13.01	1.79	52.52	
9/1	2.351	6.63	0.52	4.04	13.14	0.89	47.01	
9/8	2.324	6.79	0.47	4.00	13.60	0.28	46.49	
9/15	2.458	8.21	0.57	4.18	13.52	1.11	50.65	
9/22	2.330	7.41	0.51	4.82	12.61	0.91	51.27	
9/29	2.317	7.48	0.46	5.12	12.33	0.72	57.46	
10/6	2.156	6.79	0.63	4.55	13.00	1.12	48.72	
10/13	2.224	7.12	0.71	4.40	12.01	1.45	45.14	
10/27	2.471	7.26	0.64	4.77	14.63	0.99	55.35	

of the total ash, and 71 per cent. of the total protein to have been formed in the three-week period August 25 to September 15. The percentage of oil (figure 2) paralleled that expressed on a unit basis. The percentage of protein, however, followed a reverse curve to that expressed on a unit basis, by falling sharply during the first and the final stages of kernel development.

The decrease in the percentage of ash of the kernel, while the actual

weight increased when expressed on a unit basis, was no doubt due primarily to the rapid formation of oil, protein, and possibly carbohydrates, and not due to any significant loss of mineral constituents as will be shown. The content of ash and dry weight of the kernel was found to rise perceptibly the first few sampling periods and then to follow a gradual rise to maturity date. The phosphorus content on a unit basis as shown in figure 3 followed closely the curves given in the preceding charts for protein in the kernel, by

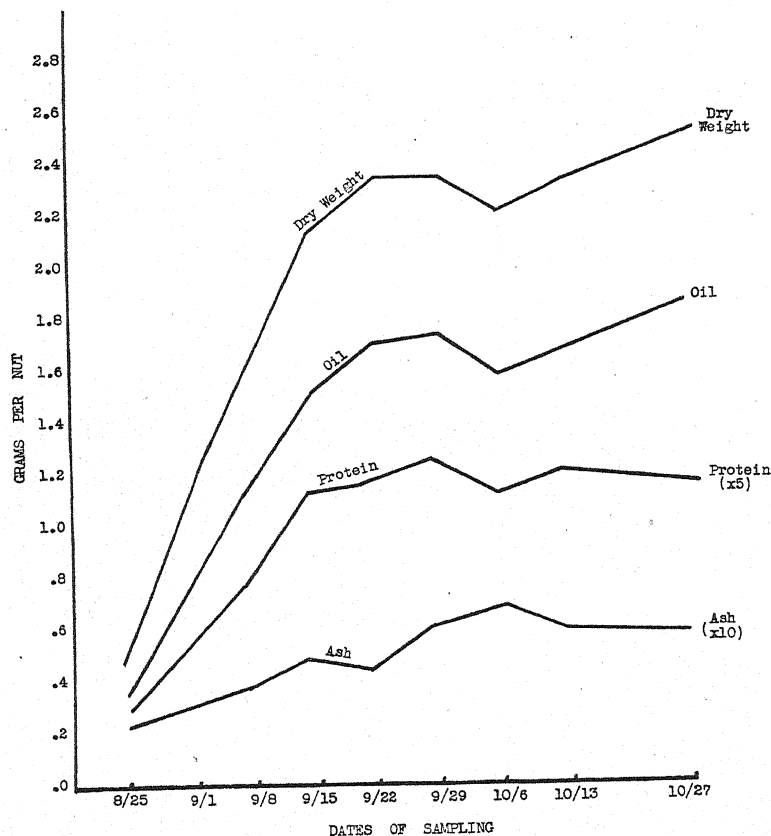


FIG. 1. Changes in dry weight, oil, protein, and ash in pecan kernels during filling. (Unit basis of grams per nut.)

rising sharply the first three weeks of sampling, and then gradually increasing in amount to maturity; while the potassium and magnesium rose more gradually to the mid-period of filling, and then declined slightly to the final stage. The percentages of phosphorus, magnesium, and calcium as shown in figure 4 remained practically the same throughout the period of filling, while that for potassium declined very sharply the first two weeks of sampling, and then leveled off to maturity. Magnesium and calcium were found to occur in the smallest amounts of the several mineral constituents in the pecan kernel.

## CHEMICAL CHANGES IN THE SHUCK AND SHELL

The content of all the mineral elements determined in the shuck and shell follow very similar curves, whether expressed as percentage or on a unit basis, as shown in figures 5, 6, 7, and 8.

The potassium content of the shuck was by far the greatest of all the mineral elements contained in the pecan nut, as shown in tables I and II. It comprised approximately 73 per cent. of the mineral elements determined.

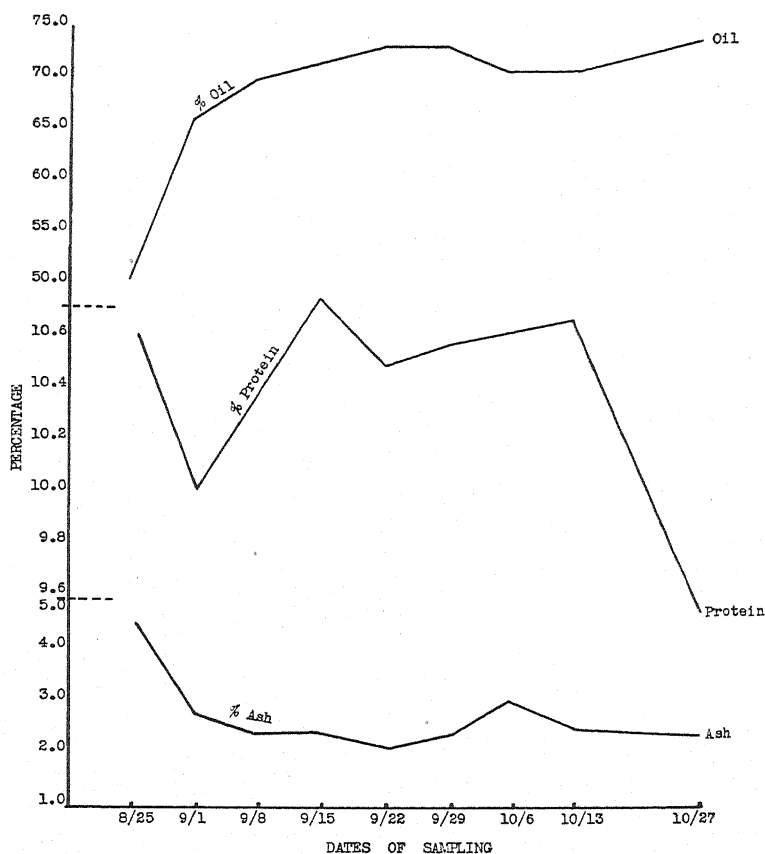


FIG. 2. Changes in oil, protein, and ash in pecan kernels during filling. (Percentage basis.)

and 34 per cent. of the total weight of the ash in the shuck. The potassium content rose rapidly in the shuck until the mid-period of filling was reached, and then declined gradually to the maturity date.

The calcium content of the shuck and shell followed somewhat similar curves, both rising sharply at the final stage of maturity. The curves for magnesium in the shell show a different picture. There was a sharp drop in magnesium in the shell during the first two weeks of sampling, and again at the final period, whereas the content of calcium increased sharply during the last two weeks. Calcium comprised more than half of the weight of the

mineral elements determined in the shell. Magnesium and phosphorus were contained in the least amounts in the shuck and shell.

#### CHANGES IN THE ASH AND DRY WEIGHT OF KERNEL, SHUCK AND SHELL

Comparisons of the dry weight and ash in the kernel, shuck and shell of the pecan are shown in figure 9. The dry weight and the weight of ash in the kernel followed parallel curves; while, calculated as percentage, the ash

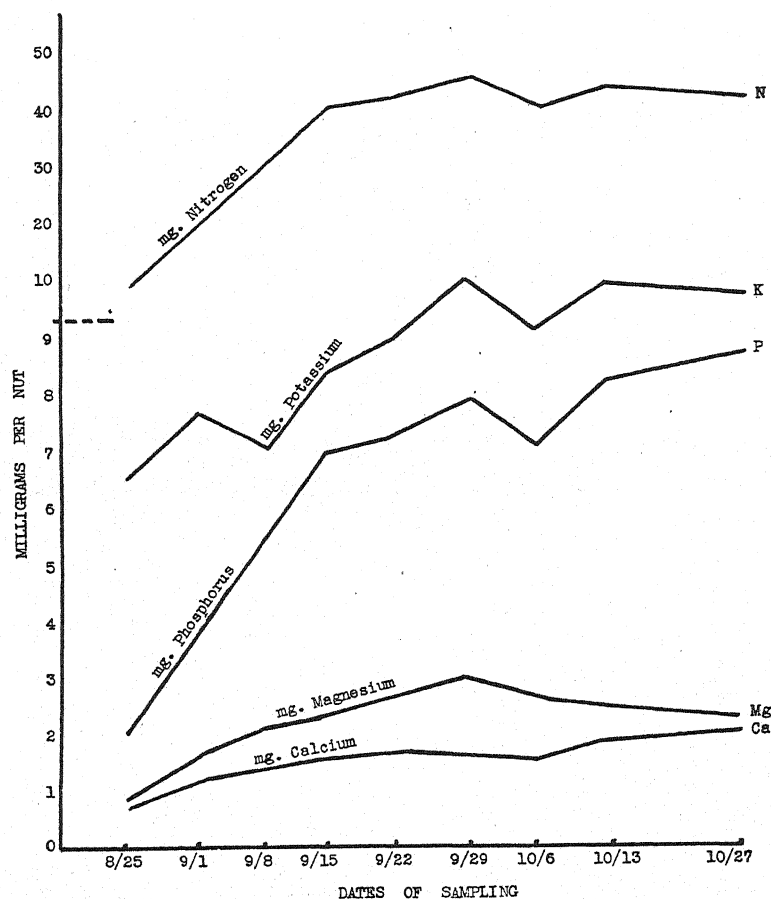


FIG. 3. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan kernels during filling. (Basis of milligrams per unit.)

followed a reverse curve. However, the ash content of the shuck and shell was found to follow almost identical curves when expressed on percentage and on unit bases. The dry weight of the kernel was found to rise sharply during the early period of filling, while that of the shuck and shell rose rapidly during the final stage of filling.

It is interesting to note (table II) that much of the dry weight and ash of the shuck and shell had already developed at the beginning of the sampling period, while only a small amount of the dry weight and ash of the

kernel was present at that time. It was found that 92.1 per cent. of the dry weight of the shuck at maturity, and 94.1 per cent. of the shell, had already been formed at the first sampling period, while only 22.4 per cent. was found to have developed in the kernel. Likewise, 70.1 per cent. of the ash in the shuck at maturity, and 94.9 per cent. of the ash in the shell, had been deposited during the stage prior to filling, while only 40.4 per cent. of the ash in the kernel had been deposited at that time.

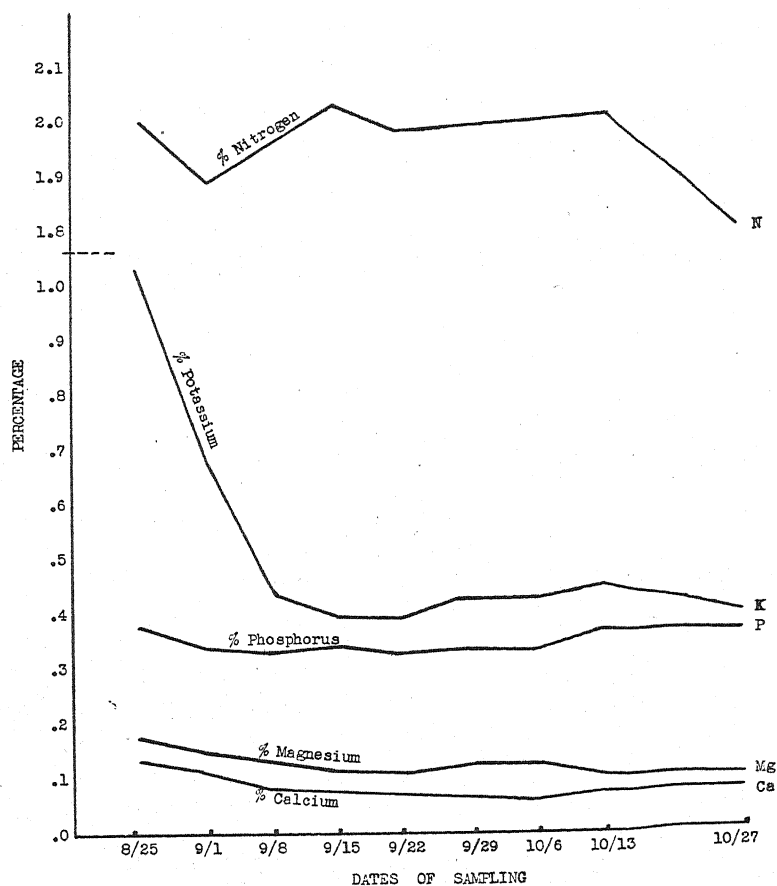


FIG. 4. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan kernels during filling. (Percentage basis.)

### Discussion

The results of oil, protein, and ash determinations in the kernel, shuck, and shell of the pecan agree closely with those found by other investigators (17, 19, 20). The data obtained by THOR and SMITH (19, 20) show that sugars are accumulated at a rapid rate in the whole fruit and shuck of the pecan during the pre-filling stage, falling rapidly soon after filling begins, and that during this pre-filling period much of the structural material has



been formed in the shuck and shell. They found that the development of the shell is practically complete at the time filling of the nuts begins, and this was borne out by ROMBERG *et al.* (13). The authors found this to be true since most of the dry weight and ash of the shuck and shell had accumulated at this time, whereas only a small portion of the weight of the kernel was evident. This bears out the conclusion of SMITH and THOR (18) that the first period of growth—the pre-filling period, prior to late August—

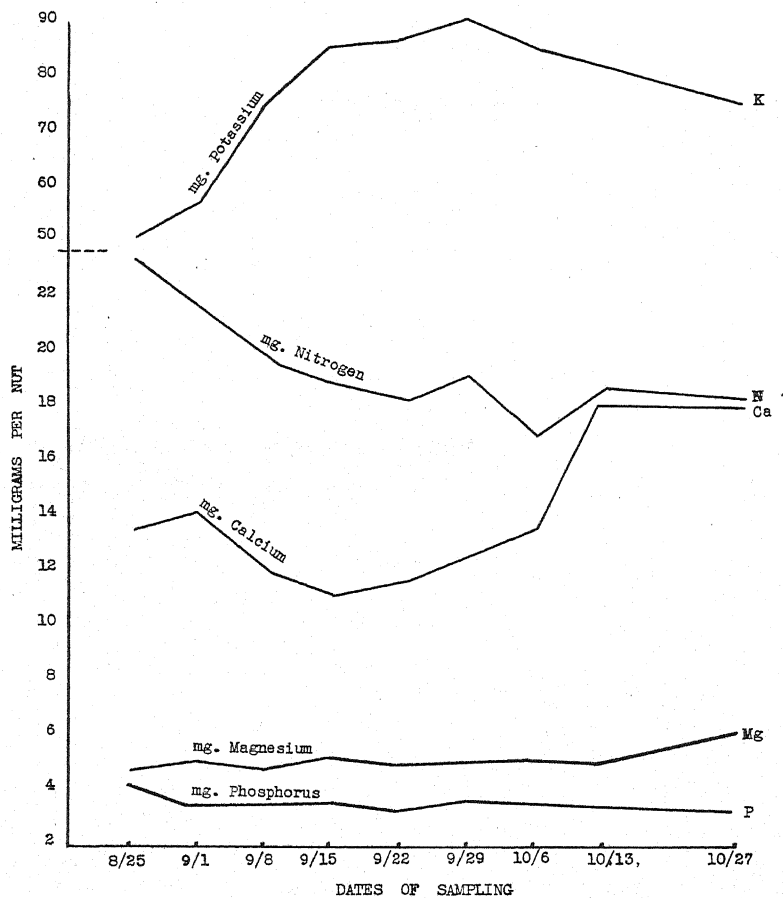


FIG. 5. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shucks during kernel filling. (Basis of milligrams per nut.)

is characterized by the formation of structural elements of the shuck and shell. During the second period, the filling of the kernel is of major importance; *i.e.*, formation of oil, protein, minerals, and acid-hydrolyzable polysaccharides. Therefore, it appears that the most important period in the development of the shuck and shell is the pre-filling period.

In the present investigation, most of the oil, protein, and mineral constituents of the kernel were found to be deposited preceding and during the

first three of the weekly periods of sampling, or before September 15, and this no doubt is the critical period in the development of the kernel.

FINCH (4) noted that by August 26, "filling" was occurring rapidly and deposition of solid materials continued for the ensuing 10 days, when the kernels were filled solidly. The marked differences obtained by calculating the data obtained during the filling of the kernel on the percentage basis and on the basis of weight per nut illustrate the important changes

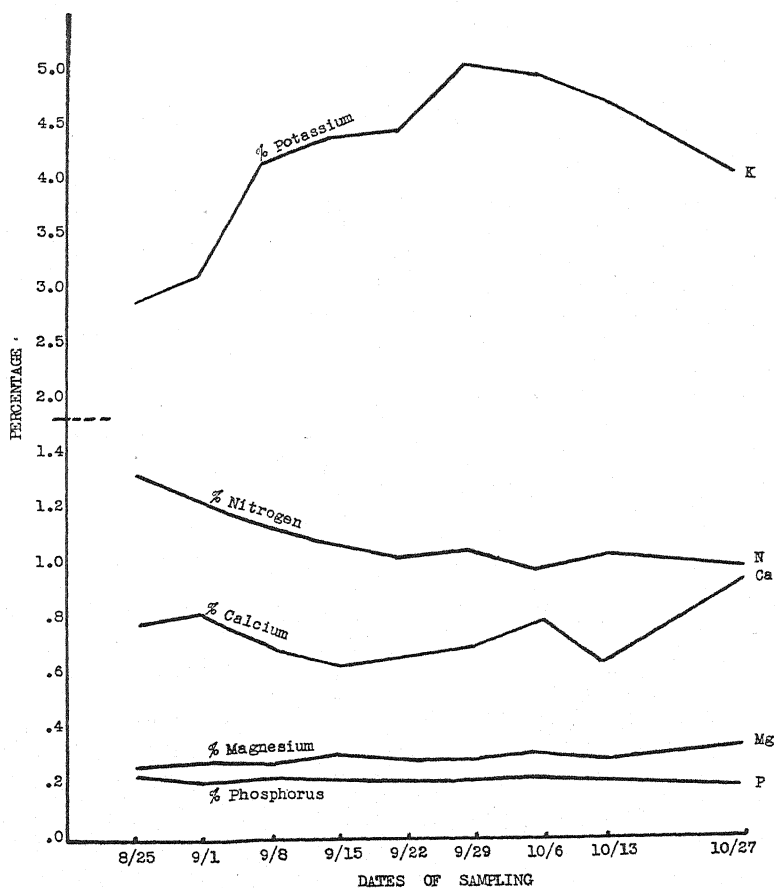


FIG. 6. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shucks during kernel filling. (Percentage basis.)

in the basic materials used in the development of the different portions of the pecan nut. The rapid formation of oil, protein, and carbohydrates in the kernel was accompanied by a similar rise in the mineral elements as shown in figure 3, but the amounts of these mineral elements were by far smaller than the amounts of the organic materials. Thus the decrease in the percentage of the ash constituents did not indicate any actual loss of these elements. On the other hand, the curves obtained by calculating the data

on percentage and on unit bases in the pecan shuck and shell were very similar, indicating that the change of materials was mainly mineral.

The changes in the chemical composition of the shuck are of utmost importance, since all the substances that enter the nut must pass through it before they can be formed in the kernel. A considerable portion of the mineral constituents of the pecan was contained in the shuck. Approximately 70 per cent. of the minerals (phosphorus, potassium, calcium, and

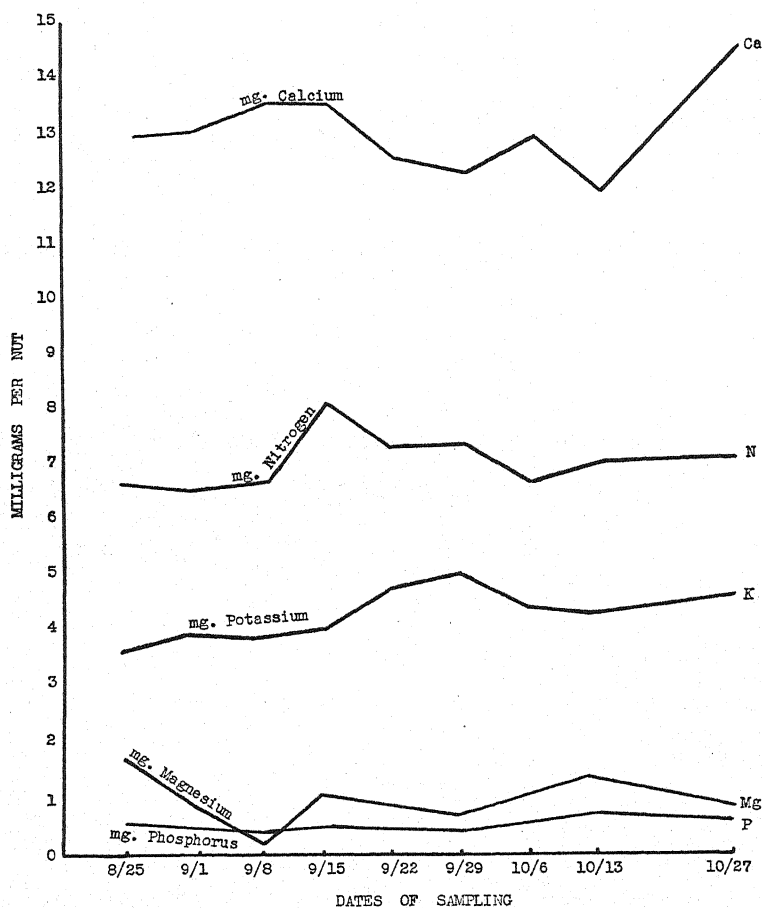


FIG. 7. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shells during kernel filling. (Basis of milligrams per nut.)

magnesium) of the mature pecan nut was contained in the shuck, while 14 per cent. was present in the shells, and 16 per cent. in the kernels.

At the time of the first sampling when the kernels were beginning to fill, the shucks contained approximately 71 per cent. of the mineral constituents, while the shells contained 19 per cent. and the kernels only 10 per cent. Thus it would seem that these chemicals perform a vital function in the filling of the kernel, either by aiding in the translocation of the materials

which go into the formation of the kernel, or directly as catalysts in the transformation into oil of carbohydrate material previously stored in the supporting shoots. The total sugar content of the pecan kernel is not built up until after the formation of oil and other constituents has stopped; and according to THOR and SMITH (18, 19, 20) and SMITH and LOUSTALOT (16, 17), this translocation is mainly from the shuck, which loses considerable quantities of sugars during the later filling stage. At the first sampling

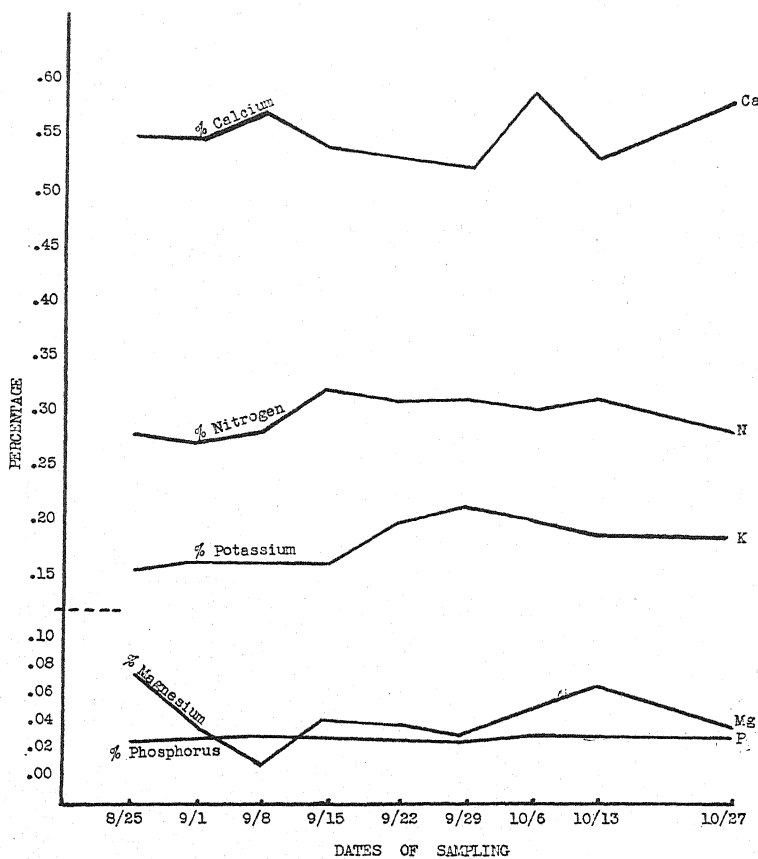


FIG. 8. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shells during kernel filling. (Percentage basis.)

period, approximately 92 per cent. of the dry weight of the shuck had been formed, but only 70 per cent. of the ash. This may be explained by the translocation of much of the sugars stored in the shucks at the beginning of the filling period, that material being translocated into the kernel during the later period of filling.

That potassium is very important in the filling processes in the pecan nut is evidenced by the large amounts present in the shuck, through which must pass all materials that go into the nut. The content of potassium increased

very rapidly in the shuck during the critical period of early filling. The gradual decline of the potassium content in the shuck after the mid-filling period had been reached indicated that potassium was transferred back into the supporting tissues, since there was no appreciable increase in the potassium content of either the shell or the kernel of the nut to account for the loss in the shuck. Another possible cause for the decline in the potassium content of the shuck during the later filling stage could have been leaching by rainfall. This, however, was very unlikely, as only 1.8 inches of rain

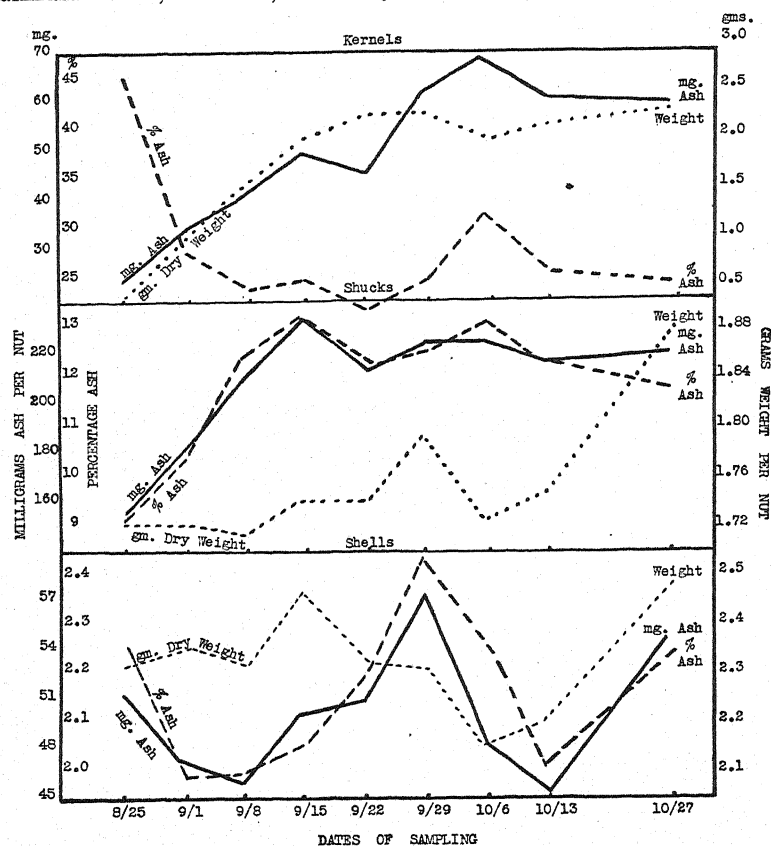


FIG. 9. Changes in dry weight and ash in pecan kernels, shucks, and shells during kernel filling. (Percentage and unit weight bases.)

were recorded in this orchard during the period in which this decline took place.

Phosphorus was found to increase rapidly in the kernel during the filling period, and no doubt performs an important function in the processes of filling. However, this element occupies only a minor position in the mineral content of the shuck and shell. The phosphorus content of the shuck declined steadily during the development of the kernel, which indicated that the filling process was drawing on phosphorus in the shuck. This,

however, would only account for about 10 per cent. of the phosphorus which went into the kernel during this period. Evidently this element was moved from the trees into the kernel with the assimilated products that go into kernel formation.

It appears that calcium is utilized mainly for the structural development of the shell, since it composed nearly 68 per cent. of the mineral constituents of the shell at the first sampling period. The shell of the pecan seems to have little or no function in the filling processes, since only minor chemical changes took place during kernel development. SMITH and LOUSTALOT (16, 17) did not find any appreciable change in any of the chemical constituents of pecan shells at different harvest dates.

### Summary

A systematic sampling of pecan nuts was made at nine weekly intervals during kernel development to determine chemical changes in the mineral constituents. Dry weight, ash, oil, nitrogen (protein), phosphorus, potassium, calcium, and magnesium were determined on the kernels, shucks, and shells and the data were calculated and reported as percentage of dry weight and on the unit basis of milligrams per nut.

Striking differences occurred in the curves representing the data expressed on percentage and on unit bases for the constituents of the kernels, but there was a similarity in such curves for the constituents of the shuck and shell. This was due primarily to the main components which were formed in the parts of the nut; those in the kernel being mainly organic, and those in the shuck and shell being for the most part mineral.

The most important stage in the development of the shuck and shell of the pecan was the pre-filling period during which 92.1 per cent. of the dry weight of the shuck and 94.1 per cent. of the dry weight of the shell had been formed, while only 22.4 per cent. of the dry weight of the kernel was evident.

The most critical period in the filling of the kernel was the period prior to September 15, at which time approximately 96 per cent. of the protein, 82 per cent. of the oil, 85 per cent. of the dry weight, and 84 per cent. of the ash of the kernel at maturity date were formed. During the three-week period from August 25 to September 15, 63 per cent. of the total dry weight, 64 per cent. of the total oil, 43 per cent. of the total ash, and 71 per cent. of the total protein were formed in the kernel, which indicated the rapidity of the changes that took place.

The potassium content of the shucks was found to be greatest of the several ash constituents in the pecan fruit, comprising 73 per cent. of all the mineral elements determined in the mature nut, and 34 per cent. of the total weight of the ash of the shuck. Potassium no doubt has a vital function in the translocation or transformation of materials stored in the kernel, since it accumulated at a rapid rate in the shuck and kernel during the early filling stage.



Data show that oil and protein were formed to a large extent in the kernel, while the main mineral constituent found in the shuck was potassium, and calcium in the shell of the pecan.

Magnesium and calcium were found to occur in the smallest amounts in the pecan kernel, while magnesium and phosphorus were lowest in the shuck and shell of the pecan.

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SOME CHEMICAL CHANGES ASSOCIATED WITH THE  
TRANSITION FROM VEGETATIVE TO REPRO-  
DUCTIVE GROWTH OF WINTER  
WHEAT<sup>1</sup>

G. R. NOGGLE

(WITH FOURTEEN FIGURES)

The physiology of development of plants has been extensively investigated during recent years. Many different plants have been studied but the results are by no means in agreement. LOEWING (7), however, has pointed out that some common relationships seem to characterize certain metabolic processes in typical annuals. Considerable information has been gathered concerning the chemical changes occurring during the transition from the vegetative to the reproductive phase of growth. The vegetative phase of growth of typical annuals is characterized by the rapid intake of water and salts, rapid organic synthesis, maximal water content, and a high respiratory level. During the reproductive phase of growth there is a general trend toward a lower water content and a reduction in the rates of respiration, photosynthesis, and protein formation in the vegetative structures.

It has been possible to exercise some control of the extent and duration of the vegetative and reproductive activities of plants with the discovery of photoperiodism and the conception of "phasic development." The investigator is able to lengthen or shorten the periods of vegetative and reproductive growth of plants by control of temperature and light. MURNEEK (8) made a biochemical investigation of the development of soybeans under short and long photoperiods. The short photoperiod was found to permit sexual reproduction of the soybeans while a long photoperiod kept the plants in the vegetative condition. The short-day plants had a greater percentage increase of dry matter and a higher nitrogen concentration than the long-day plants. The carbohydrate concentration was relatively lower in short- than in long-day soybeans during the first 15-16 days of growth. When young soybean plants were switched from one photoperiod to another, within 9-10 days the concentration of nitrogen and carbohydrate changed so that the resulting concentration was typical of that obtaining under a similar but continuous exposure to the new length of day. The orientation was complete physiologically and morphologically in 17 days. HIBBARD (4) investigated the changes in activity of catalase, peroxidase, invertase, amylase, and reducase in soybeans under short and long photoperiods. He observed that changes in enzymatic activity could be detected as early as five days after the beginning of the treatments, and that these differences in enzymatic activity could be detected prior to any morphological or chemical changes.

<sup>1</sup> The expenses incurred in the present study were borne in part by a grant from the Cereophyll Laboratories, Inc., Kansas City, Missouri.

The object of the present study was to determine how soon changes in concentration of certain constituents could be detected in vernalized winter wheat when it was switched from a vegetative photoperiod to a reproductive photoperiod.

### Methods

The seeds of a winter wheat (Brill—selection from Turkey Red) were soaked overnight and then placed on clay germinating blocks and held at 2° C. for sixty-seven days. The seeds germinated and the seedlings developed two leaves during the vernalization period. The seedlings were transferred to pots of soil and placed under a daily photoperiod of eight hours. The greenhouse temperature was maintained at 65–70° F. The soil moisture in the pots was maintained at 50 per cent. of the water-holding capacity by weighing the pots on alternate days and adding the water lost by transpiration and evaporation.

Samples were cut at two- to four-day intervals while the plants were on the short-day photoperiod. After the switch-over from short- to long-day, samples were cut daily. All samples were cut at 1:00 P.M. and the green weight of the leaves, stems, and tillers determined. Ascorbic acid and chlorophyll were determined on a composited sample containing fresh leaves, stems, and tillers. Dry weight was determined separately in leaves, stems, and tillers. The samples were then ground in a Wiley mill and stored for chemical analysis.

The ascorbic acid was extracted in a Waring blender with 0.5 per cent. oxalic acid and determined colorimetrically by the method of LOEFFLER and PONTING (6). Chlorophyll was determined by the method of COMAR, BENNE, and BUTEYN (1). Riboflavin was assayed microbiologically using *Lactobacillus casei*. The micro-Kjeldahl procedure was used to determine the total nitrogen content.

### Data and discussion

The eight-hour photoperiod was not adequate to permit the plants to enter the reproductive phase of development. The growing point remained undifferentiated and new leaves were formed until the plant had nine leaves. The plants were then transferred to a daily photoperiod of fourteen hours. Within ten days the plants showed evidences of jointing and the growing point differentiated floral primordia.

During the course of the experiment, measurements of the height of the plants were taken at weekly intervals (fig. 1). Several periods of change in growth rate were observed following the switch-over from short- to long-days. These changes in growth rate are especially evident when the weekly increments of increase in height are plotted as shown by figure 2. There was a considerable increase in height immediately following the switch-over. During the following week there was little increase in height of the plants. The period probably was one of considerable internal readjustment both morphologically and physiologically. Another period of increased growth

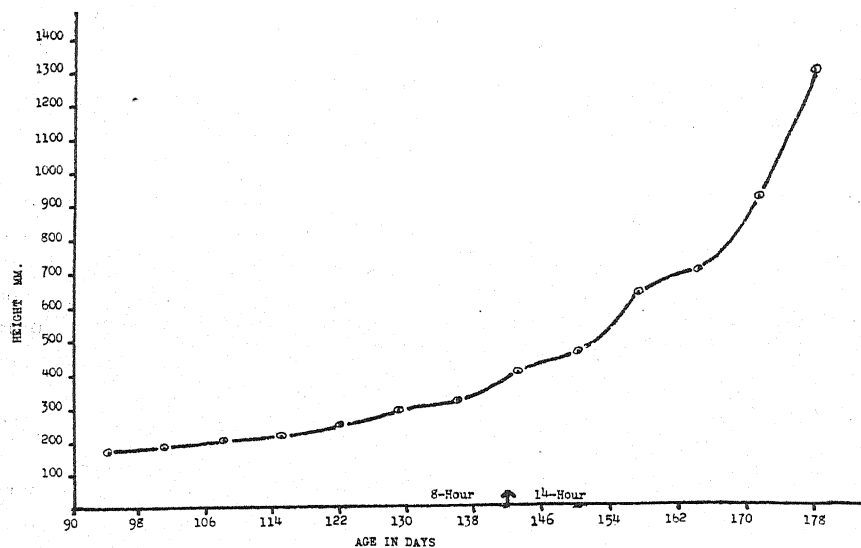


FIG. 1. Total height of plants at weekly intervals.

was evident during the following week. The increase in height was associated with the elongation of the internodes and lengthening of the stem. The next week was characterized by a decreased growth increment. During this week the boot leaf developed and the spike formed. There was but little

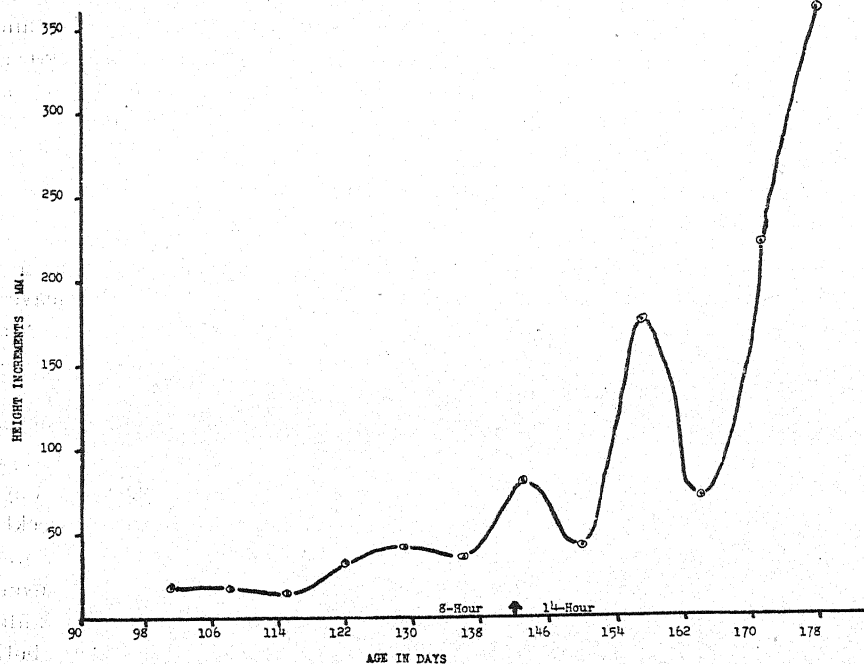


FIG. 2. Weekly increments of height.

increase in height due to stem elongation. The plants steadily increased in height due to the emergence of the spike and the elongation of the grain stalk during the following weeks.

The total green weights of the wheat plants are shown in figure 3. There was a drop in the total green weight two days after the plants were switched from short- to long-days. During subsequent samplings, there were several other fluctuations in green weight, but the general trend remained toward an increased green weight. Figure 4 shows the green weight of leaves and stems. The leaves showed considerable decrease in green weight after the switch-over. The stem appeared after the plants were exposed for several

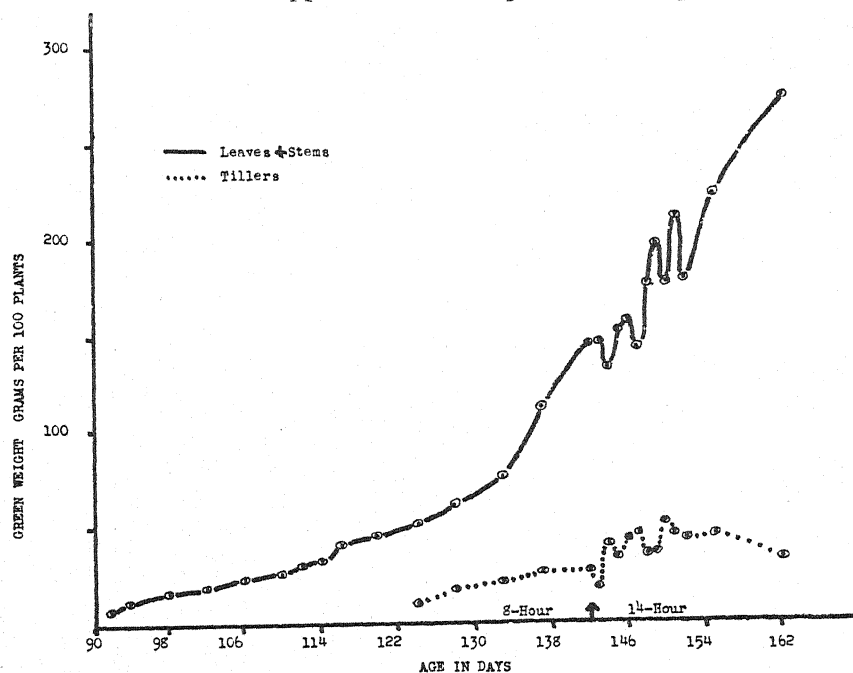


FIG. 3. Green weights of plants (leaf and stem) and of tillers.

days to the long photoperiod and soon dominated the total green weight of the plant.

The total dry weight, as seen in figure 5, showed some irregularities following the switch-over from short- to long-days. From the data presented in figure 6, it is seen that there was an immediate decrease in dry weight of leaves after the switch-over. The dry weight of the leaves recovered and then increased. The stems showed two periods of slow increase in dry weight followed by a period of rapid dry weight production.

The initial decrease in green weight production following the switch-over was probably due in part to a decreased moisture content. An examination of the growing point during this period indicated that flower primordia were being differentiated. LOEWING (7) has pointed out that a loss of tissue



moisture is characteristic in annuals at the time of initiation of flower buds. However, some of the wide irregularities in green weight might be due to sampling differences. The decrease in dry weight production of the leaves was associated with the increase in dry weight of the stems. It is likely that translocation of various constituents took place from leaves to stems and accounted for the decrease in dry weight of the leaves and increase of the stems.

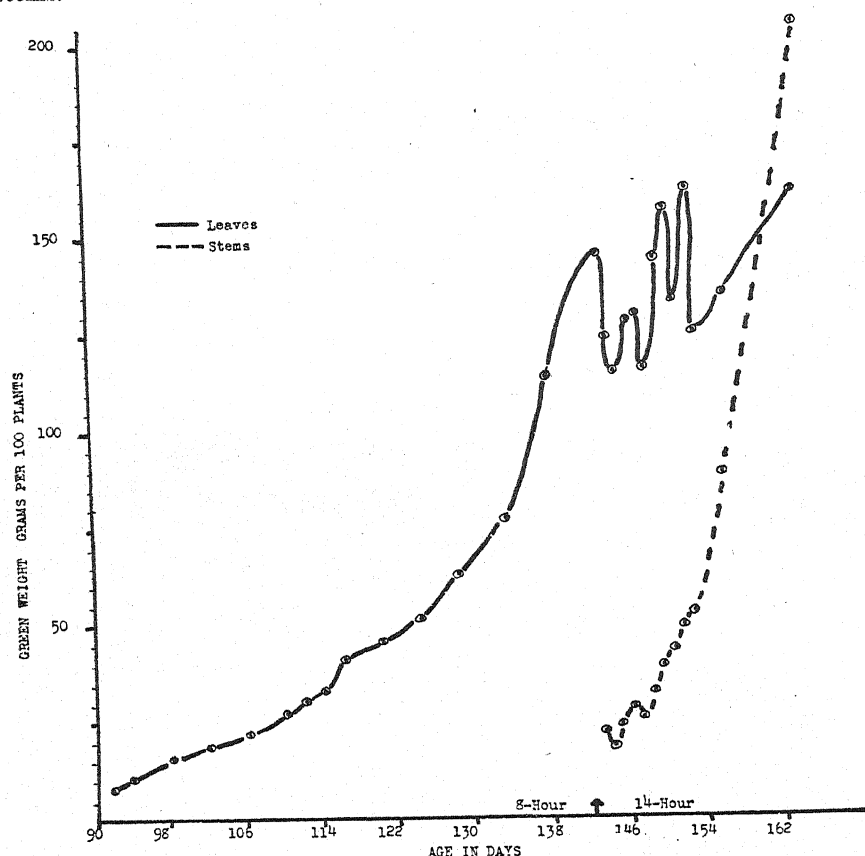


FIG. 4. Green weights of leaves and stems.

The weekly increments of green and dry weight production of the total plant are shown in figure 7. During the period immediately following the switch-over the green weight production was at a standstill. This period of decreased production was followed by two periods of accelerated green weight production. The increments of dry weight do not indicate any such decreased production as was noted in the green weight curve. This situation suggests that the moisture in the tissue was the constituent most seriously affected. Some deviations in the dry weight increment curve might have been found if different sampling dates had been used.

WITTWER (11) demonstrated that several periods of accelerated growth

of corn were associated with synapsis and syngamy. He found that increased growth hormone production during these periods was responsible for the accelerated rates of growth. In the present experiment, synapsis was observed but syngamy did not occur. The fluctuations in the growth are likely associated with some phase of the synaptic process but the nature of the relationship is not apparent.

A high concentration of total nitrogen was maintained in the young wheat plant as shown by figure 8. The nitrogen concentration dropped off

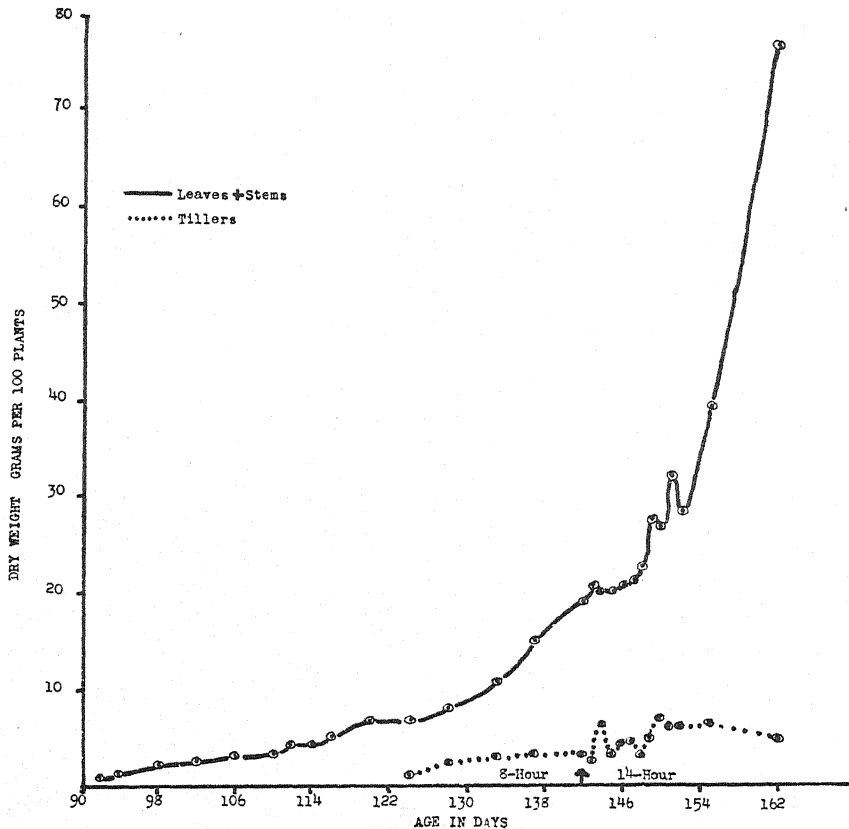


FIG. 5. Dry weights of plants (leaf and stem) and of tillers.

in the later vegetative stages and following the switch-over from short- to long-days the per cent. nitrogen rapidly decreased. The regular fluctuations in the percentage of nitrogen which followed the switch-over are difficult to explain. The dry weight of the plant did not show such fluctuations, but the points of maximum nitrogen concentration occurred on days when the leaves had a high green weight or moisture content.

The green weight of the stems did not show fluctuations similar to those noted in the leaves. The nitrogen content, however, of the stems followed that of the leaves. This would indicate that the nitrogen was being synthe-

sized in the leaves and rapidly translocated to the stems. The parallel fluctuations of nitrogen in leaf and stem suggest that the altered photoperiod has induced internal metabolic changes, the nature of which are unknown. The green and dry weights of the tillers showed little similarity to the green and dry weights of the leaf or stem tissue but the fluctuations in nitrogen content of the tillers followed closely those noted for leaf and stem. The similarity of nitrogen changes in leaf, stem, and tiller suggests that the nitrogen is related to some internal metabolic disturbance set off by the change in photoperiod.

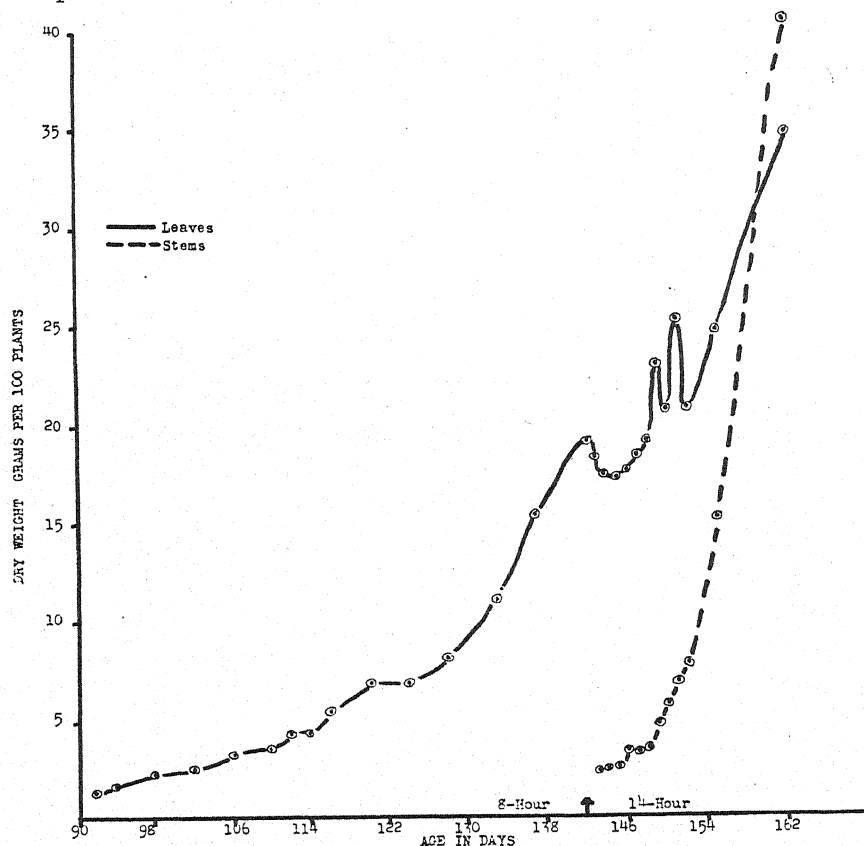


FIG. 6. Dry weights of leaves and stems.

The riboflavin concentration of the plant tissue is shown in figure 9. The riboflavin showed an early maximum concentration and then diminished steadily as the plant matured. Following the switch-over from short- to long-day photoperiod the concentrations of riboflavin varied similarly to the total nitrogen concentrations. There was one difference, however, in that immediately following the switch-over the nitrogen content sharply dropped and then recovered on the 145th, 148th, and 151st days while the riboflavin concentration did not decrease following the switch-over but sharply in-

creased in concentration on the 145th, 148th, and 151st days. Leaves, stems, and tillers showed trends in riboflavin concentration identical with those noted for the nitrogen content of the leaves, stems, and tillers. The similarity of behavior of the nitrogen and riboflavin suggests that the two constituents are related to some internal metabolic changes brought about by the altered photoperiod.

Figure 10 shows that the ascorbic acid concentration of the fresh tissue fluctuated widely during the growing period of the plant. The trend was

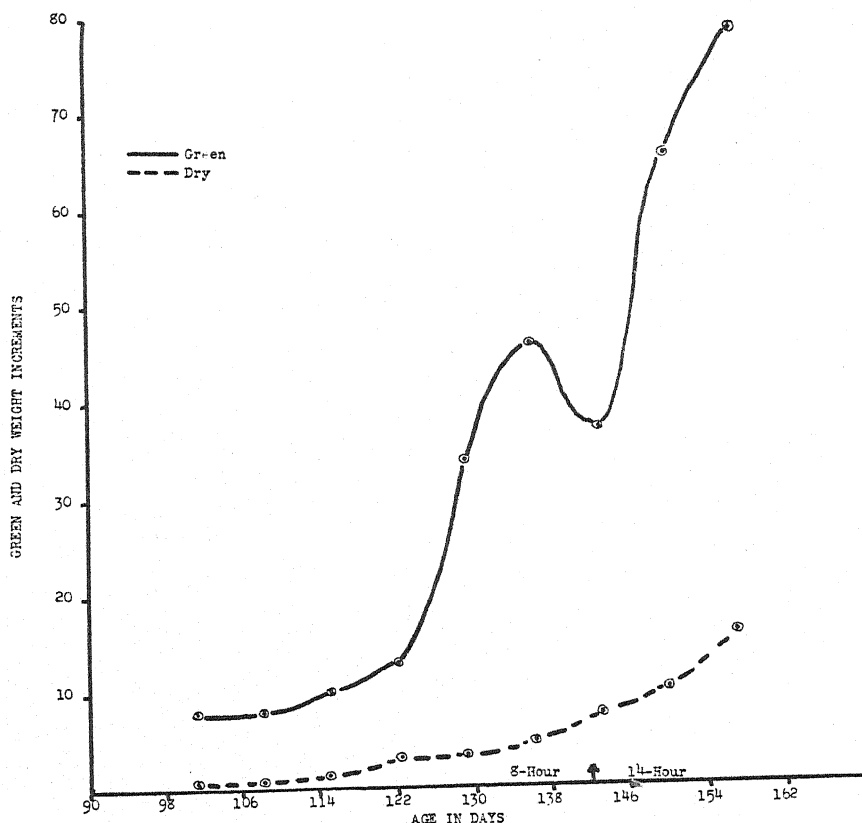


FIG. 7. Weekly increments of green and dry weight.

toward a decreased concentration of ascorbic acid as the plant matured. Following the switch-over, the ascorbic acid content attained three peaks in concentration on the 144th, 147th, and 150th days. These peaks in ascorbic acid concentration coincided with days on which the green weight of the plant was at a minimum. It was pointed out that the fluctuations in green weight were mainly due to fluctuations in the moisture content of the tissue. It thus appears that the ascorbic acid concentration is dependent upon the degree of hydration of the plant tissue. That the degree of tissue hydration is not entirely the cause of the fluctuations in ascorbic acid concentration

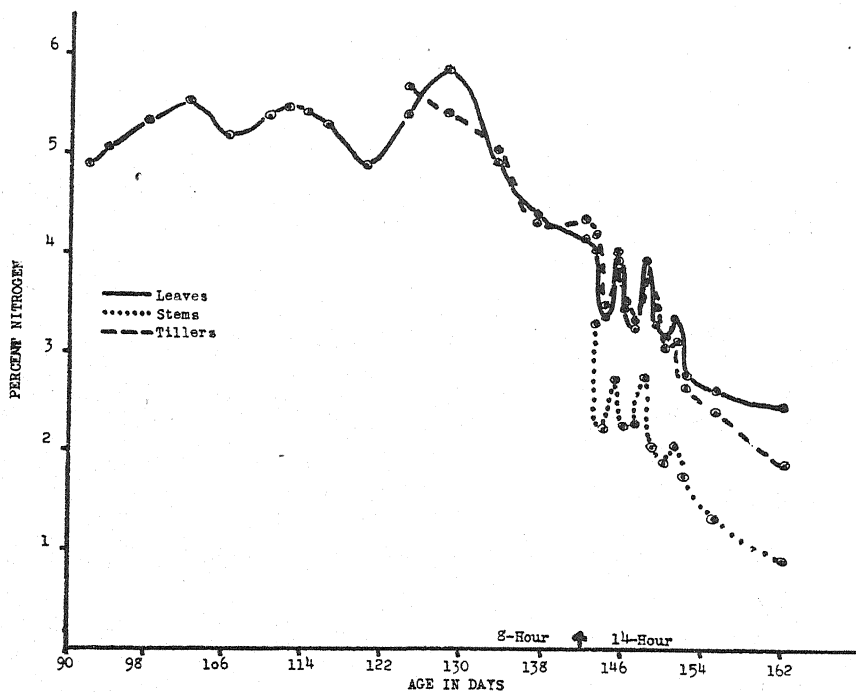


FIG. 8. Percentage of nitrogen in dry samples of leaves, stems and tillers.

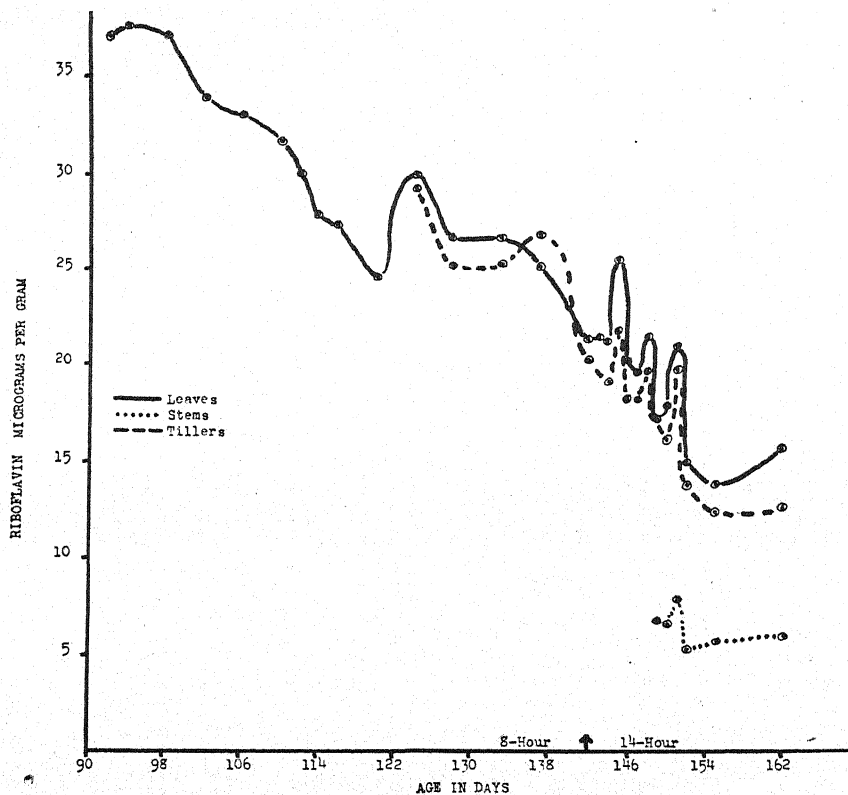


FIG. 9. Micrograms of riboflavin per gram of dry tissue of leaves, stems, and tillers.

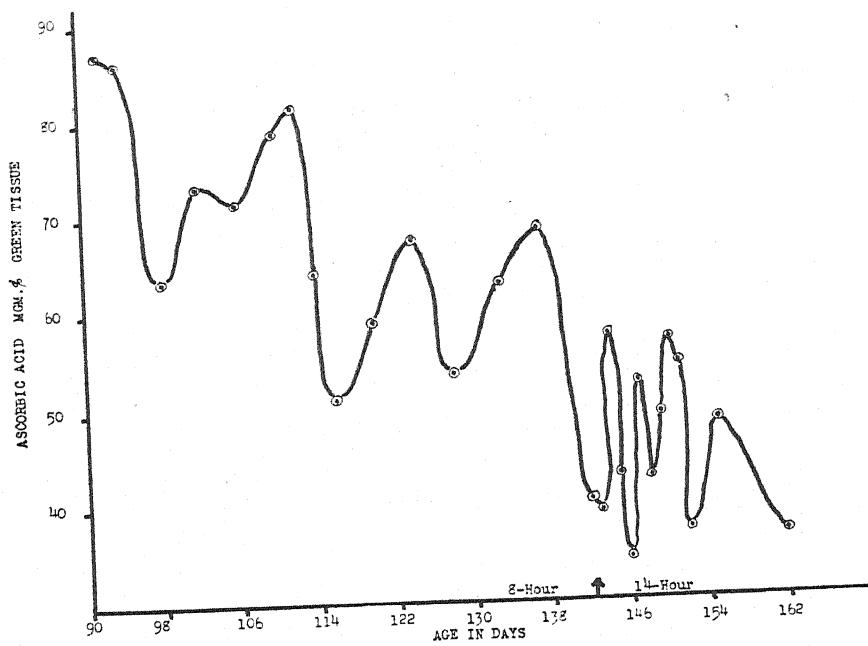


FIG. 10. Milligrams of ascorbic acid per 100 grams of fresh plant tissue.

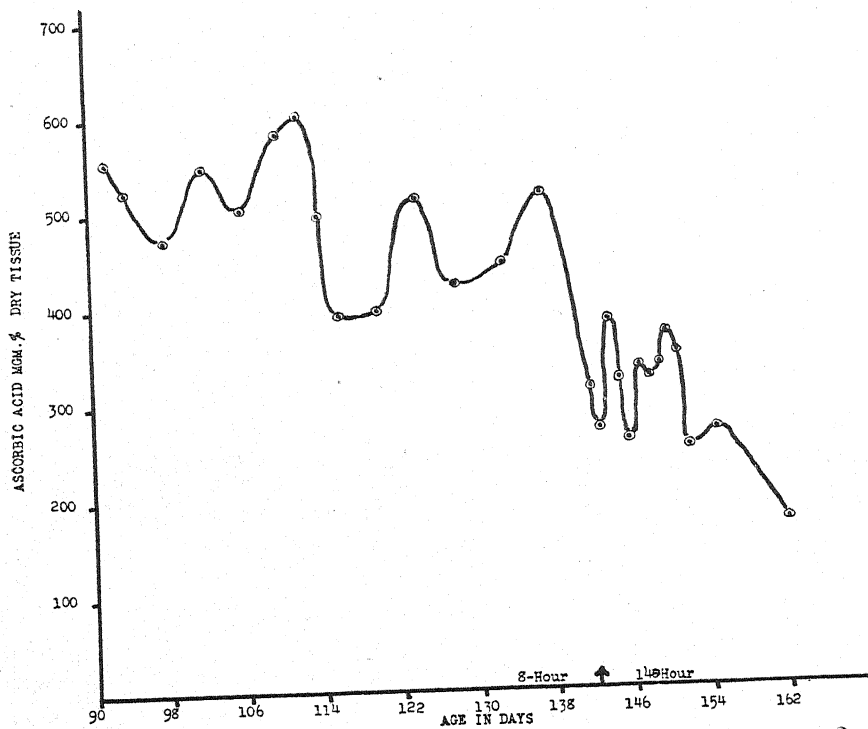


FIG. 11. Milligrams of ascorbic acid per 100 grams of dry plant tissue.



can be seen from figure 11, where the ascorbic acid has been calculated on a dry weight basis. The fluctuations in ascorbic acid concentration did not coincide with the fluctuations noted for nitrogen and riboflavin contents. The peaks in ascorbic acid concentration occurred one day earlier in each case than the peaks in concentration of nitrogen and riboflavin. It appears that the internal processes in plant metabolism influenced the ascorbic acid concentration in a different manner than the nitrogen and riboflavin concentration.

A number of investigations (3, 10) have shown that the ascorbic acid concentration of plant material is dependent upon the amount and duration of

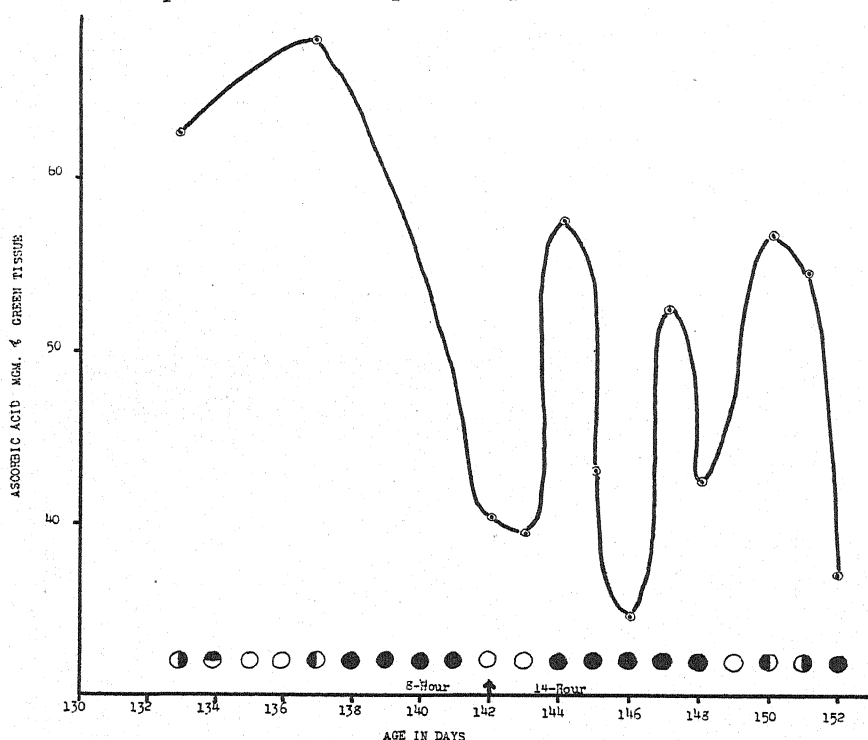


FIG. 12. Influence of daily amounts of sunlight on the ascorbic acid concentration of plant tissue. ○ = Sunlight all day; ● = cloudy all day; circle with right half shaded = morning clear, afternoon cloudy; circle with left half shaded = morning cloudy, afternoon clear; circle with top half shaded = intermittently clear and cloudy.

light that the plants receive. A daily record was kept of the cloudy and sunny days during the course of the experiment and is shown in figure 12. One might possibly account for several of the fluctuations in ascorbic acid concentration by the amount of sunlight that the plants received, but several of the fluctuations were independent of the durations of sunny or cloudy days. The data suggest that the ascorbic acid is connected with certain phases of metabolic activity in the plants.

The chlorophyll concentration of the fresh tissue is shown in figure 13.

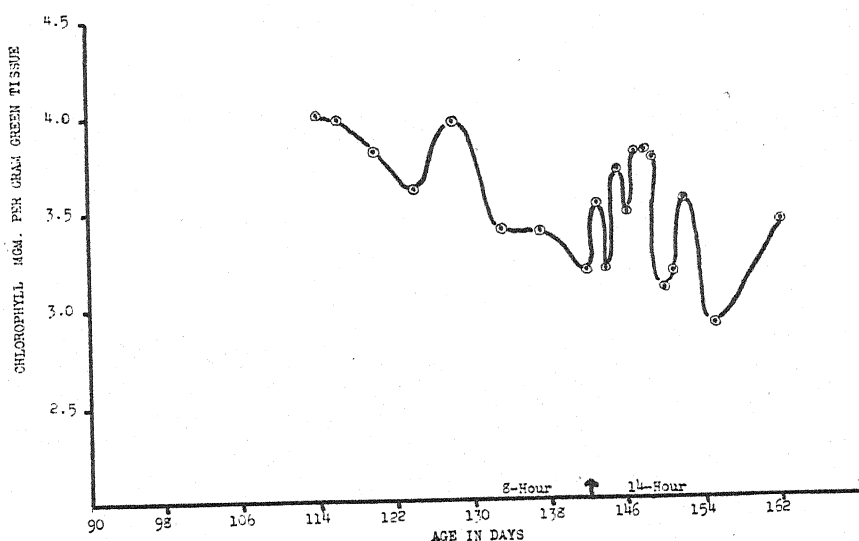


FIG. 13. Milligrams of chlorophyll per gram of fresh plant tissue.

With the exception of the high chlorophyll concentration on the 128th day, the chlorophyll decreased as the plant matured. No explanation is suggested for the high chlorophyll concentration on the 128th day, but the nitro-

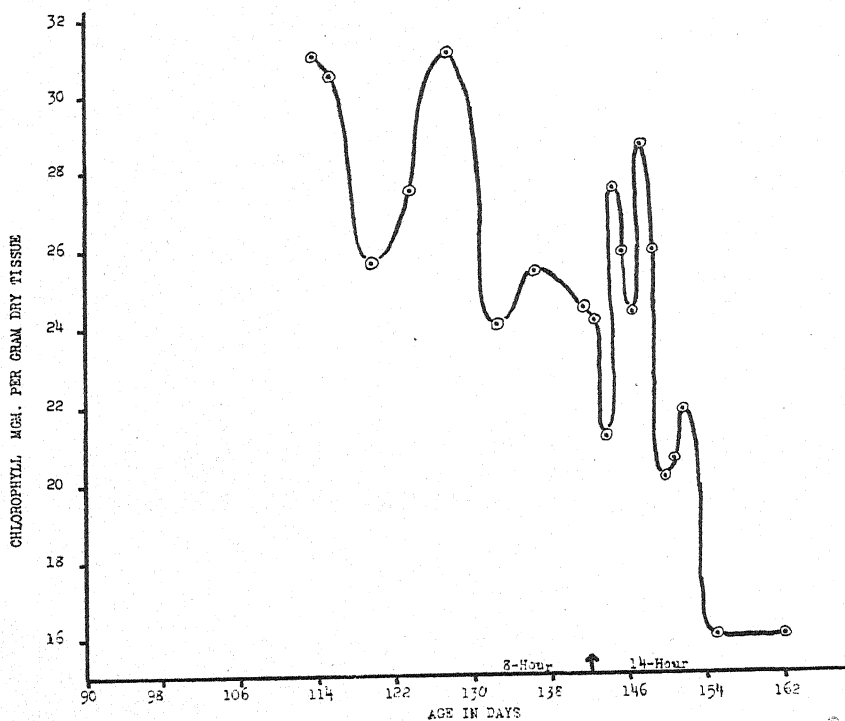


FIG. 14. Milligrams of chlorophyll per gram of dry plant tissue.

gen concentration also showed a peak on this day. Following the switch-over from short- to long-day, the chlorophyll concentration tended to increase in magnitude. Several workers (2, 8, 9) have pointed out that a high chlorophyll and carotene content is associated with reproductive activity. No carotene determinations were made, but it can be seen that the chlorophyll concentration of the plant tissue increased when the plant entered the reproductive phase of development. Undoubtedly, some of the fluctuations in chlorophyll concentration can be attributed to differences in moisture content of the tissue. If the chlorophyll concentration is calculated on a dry-weight basis, as shown by figure 14, several interesting facts are observed. The peaks of chlorophyll concentration on days 145 and 148 coincide with the peaks in nitrogen and riboflavin concentration. The chlorophyll concentration then rapidly declined except for a small increase on the 151st and 152nd days. The decreased chlorophyll concentrations in the later stages of growth were caused by the disappearance of chlorophyll from the lower dead leaves of the plants.

The data presented above suggest that nitrogen, riboflavin, and chlorophyll behave similarly during the transition from vegetative to reproductive growth. The ascorbic acid does not seem to be directly related to these constituents. Nitrogen and riboflavin are known to be high in tissues with a high respiratory activity (5, 7). Chlorophyll and carotene have been suggested to be associated with the reproductive phase of growth (2, 8, 9). It appears that the changed photoperiod has induced reproductive growth accompanied by a high level of metabolic activity.

The present state of knowledge regarding the functions of such constituents as nitrogen, riboflavin, ascorbic acid, and chlorophyll is rather meager. It has been demonstrated in this experiment that these constituents changed in concentration when the plant was switched from conditions of vegetative growth to conditions of reproductive growth. Altered rates of growth, as measured by total height, green and dry weight, also occurred when the plants were switched from vegetative to reproductive conditions. A more detailed determination of such constituents as carbohydrate fractions, nitrogen fractions, minerals, enzyme systems, and growth hormones will have to be made before a complete picture can be obtained of the chemical changes that accompany the morphological development of plants as they go from the vegetative to the reproductive phase of growth.

### Summary

1. Winter wheat was vernalized at 2° C., planted in pots of soil, and then grown under a daily eight-hour photoperiod.
2. The short-day photoperiod permitted only vegetative growth.
3. Following a switch-over to a long-day photoperiod, the plants jointed and produced grain.
4. There were several rhythmic fluctuations in concentration of nitrogen, riboflavin, ascorbic acid, and chlorophyll following the switch-over from short- to long-day conditions.

5. The nitrogen, riboflavin, and chlorophyll behaved similarly following the switch-over.

6. The fluctuations in ascorbic acid concentration occurred a day earlier than the fluctuations in concentration of nitrogen, riboflavin, and chlorophyll.

7. The changes in concentration of nitrogen, riboflavin, ascorbic acid and chlorophyll were detected before external evidences of flowering were evident.

The author is indebted to Dr. F. L. WYND for his advice during the course of this research.

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## RESPIRATION OF COTTONSEED

M. L. KARON AND A. M. ALTSCHUL

(WITH FIVE FIGURES)

### Introduction

MALOWAN (12, 13), in an investigation of the chemical and physical changes that take place in cottonseed during storage, observed that when the moisture content of prime cottonseed was artificially raised, its rate of respiration was also increased. When moist seeds which had a high rate of respiration were put in thermally insulated containers, a rise in the temperature of the seeds was noted. The highest temperature observed under Malowan's experimental conditions was 157° F., although he had noted temperatures up to 175° in carloads of seeds received at oil mills. BAILEY and coworkers (3, 4, 5) have extensively studied the respiration and storage behavior of cereal grains. RAMSTAD and GEDDES (15) investigated the behavior of soybeans in this respect. LARMOUR *et al.* (10) investigated the respiration of sunflower seed and flaxseed.

These and other investigations revealed several generalizations which may be recapitulated as follows:

1. Under carefully controlled experimental conditions, the respiration intensity of seeds, as measured by their rate of carbon dioxide production, is a regular function of their moisture content. The curves obtained by plotting respiration intensity against the moisture content of the seeds are logarithmic in character. This relationship between moisture content and respiration intensity holds, however, only when all the experiments are made on samples of the same lot of seeds and when each respiration measurement is made exactly the same length of time after the seeds are conditioned.

2. Broken and shriveled seeds, kernels, and flakes have higher respiration intensities than clean, intact seeds of the same type and moisture content.

3. The rate of respiration of seeds varies with respect to a number of factors, such as length of exposure to moisture, age, variety, conditions of growth, and other indeterminate properties. It is not possible, then, on the basis of moisture content alone, to predict respiration intensity. Similarly, it has been previously shown (8) that it is impossible to predict the rate of free fatty acid production in cottonseed on the basis of moisture content alone.

4. Oilseeds respire at a higher rate than do cereal grains of the same moisture content.

The purpose of the present investigation has been to determine the effect of such variables as moisture content, variety, location of growth, and age on the respiration of cottonseed. For convenience in expressing the effect of these variables in a quantitative manner, a quantity designated "average respiration intensity" (RI) has been proposed, and a means for its measurement has been developed.

## Description of the cottonseed samples

The samples used in these experiments were of three different varieties of cottonseed grown at three widely separated Agricultural Experiment Stations. Further diversity was introduced by the circumstance that samples of the same variety were taken at different times during the cotton-harvesting season. Thus, a wide cross section of cottonseed, differing as to variety, locality, and date of harvesting, was available for investigation.

The seed samples used in the respiration experiments are described in table I. Those designated as 104, 204, and 304 were from bolls which had

TABLE I  
DESCRIPTION OF SAMPLES OF *Gossypium hirsutum* USED IN THE  
RESPIRATION INVESTIGATIONS

SAMPLE	VARIETY	DATE PLANTED	DATE OF HARVESTING	FREE FATTY ACID CON- TENT	MOIS- TURE* CON- TENT
104	Delfos 3506†	.....	Aug. 29, 1942	1.48	12.0
105	"	.....	Oct. 3, 1942	0.90	10.6
204	Coker's 200, strain 1‡	April 20, 1942	Sept. 22, 1942	7.12	13.7
205	"	"	"	0.99	10.2
206	"	"	Oct. 22, 1942	0.76	10.0
304	Oklahoma Triumph, 0-52-12§	.....	Sept. 22, 1942	1.62	10.5
305	"	.....	Oct. 1, 1942	1.04	10.1
306	"	.....	Oct. 28, 1942	1.04	10.6
307	"	.....	Nov. 28, 1942	0.85	9.3
308	"	.....	Dec. 28, 1942	0.81	15.7

\* This refers to the percentage of moisture of the seeds as received, on a wet basis.

† Grown at Stoneville, Mississippi.

‡ Grown at Clemson, South Carolina.

§ Grown at Stillwater, Oklahoma.

just begun to crack open, being, therefore, typical seeds from an early or premature harvest of cotton. The next series of samples (105, 205, and 305) was taken from a normal harvest of cotton and the remaining samples were from cotton that had been kept in the field from one to three months after maturation before being harvested.

It will be observed that natural variations existed in the moisture contents of the seed samples as received in the laboratory. In addition, portions of all samples were treated so as to artificially change the moisture content to values above or below the natural condition. It was thus possible to measure the effect of moisture on seed properties over a wide range of natural and artificially induced moisture content.

## Experimental method

Three-pound lots of seed were set aside for each series of measurements. Whenever it was necessary to change the moisture content artificially either



above or below its original value, care was taken to so condition the seeds to the desired moisture content that any resulting biochemical activity would be minimized and permanent injury to the seeds would be prevented. Conditioning was accomplished in this manner by carrying out all moisture changes in a cold room, as previously described (8). Each 3-pound lot of seeds was then stored in air-tight jars at 25° C.

In order to measure the respiration of the seeds in any one lot, a 10-, 25-, or 50-gram sample was withdrawn from the storage container and placed in a stoppered respiration flask (1) for a length of time considered sufficient to produce a significant change in the atmosphere in the flask. A sample of gas, withdrawn from the flask, was then analyzed for oxygen and carbon dioxide by means of the apparatus and procedure described in a preceding publication (1). Upon completion of the analysis, the seed sample in the respiration flask was discarded, and fresh samples were used for subsequent respiration measurements, the size of the sample and the length of the respiration interval being based on the results of the previous analysis. Thus, for example, when measurements indicated a rising rate of respiration for a lot of seeds, the size of the subsequent samples and the length of the respiration testing intervals were reduced accordingly.

#### RESPIRATORY QUOTIENT

Both oxygen and carbon dioxide were measured in each respiration analysis, thus making it possible to calculate the ratio of carbon dioxide evolved to oxygen absorbed, or the respiratory quotient (RQ), in resting cottonseed. Complete respiration patterns were obtained on 17 different

TABLE II

THE VARIATION OF THE RESPIRATORY QUOTIENT (RQ) OF COTTONSEED  
DURING STORAGE

SAMPLE .....	105e		205e		307a	
MOISTURE CONTENT .....	16.8%		15.9%		12.9%	
	LENGTH OF STORAGE	RQ	LENGTH OF STORAGE	RQ	LENGTH OF STORAGE	RQ
	<i>days</i>		<i>days</i>		<i>days</i>	
	15	0.96	46	0.92	15	0.91
	49	1.08	91	0.93	51	0.94
	79	0.99	116	0.99	86	0.97
	130	0.98	144	1.05	162	0.96
	146	1.00	.....	.....	.....	.....
	167	0.94	.....	.....	.....	.....
	192	1.12	.....	.....	.....	.....
	239	0.85	.....	.....	.....	.....
AVERAGE RQ .....	.....	0.99	.....	0.97	.....	0.95

lots of cottonseed, in none of which was there any regular progression of the value of the RQ during the course of the storage period. Instead, all the fluctuations observed were of a random nature, as illustrated by the ex-

amples given in table II. The values of the RQ for each lot of seeds were, therefore, averaged over the complete storage period, with the results shown in table III.

It is quite evident from an examination of these results that the value for the respiratory quotient of resting cottonseed is very nearly unity and is independent of moisture content, variety, place of growth, and harvesting period. Seeds with artificially adjusted moisture contents gave results identical with those allowed to retain their natural moisture contents.

Inasmuch as the RQ of resting cottonseed was found to be unity, it thus becomes possible to determine its respiration by means of a single measurement of either the carbon dioxide evolved or the oxygen absorbed. The

TABLE III

THE AVERAGE RESPIRATORY QUOTIENT (RQ) OF STORED COTTONSEED SAMPLES

SAMPLE	MOISTURE CONTENT	RESPIRATORY QUOTIENT	GROUP AVERAGE
	%		
104b	10.0	0.95	1.00
105b	10.7	1.02	
104a	12.0	1.01	
105a	13.1	1.05	
105f'	14.9	1.00	
105e	16.8	0.99	
204b	12.5	0.95	0.98
205a	12.5	1.04	
204a	13.7	0.98	
206a	14.7	0.93	
205e	15.9	0.97	
305b	10.1	1.01	1.00
306b	10.6	0.96	
307a	12.9	0.94	
306a	13.1	1.02	
304a	13.3	1.09	
308a	15.7	0.95	
Average respiratory quotient .....			0.99

respiration data in this publication, therefore, are presented in terms of carbon dioxide evolution, it being understood that the amount of oxygen absorbed is equivalent to the amount of carbon dioxide evolved.

Simply interpreted, a respiratory quotient of unity indicates that carbohydrate is being metabolized in the respiration process. Cottonseed kernels contain approximately 14 per cent. carbohydrates, of which 8 per cent. is soluble carbohydrates (14). Thus, there is sufficient carbohydrate in the seeds to sustain the respiration that takes place during the storage period. MALOWAN (13) has actually demonstrated that there is a measurable decrease in carbohydrate content of cottonseed after the seeds have heated in storage. This observation is paralleled in work by RAMSTAD and GEDDES (15), who, on the basis of their calculations of the specific heat of resting soybeans, concluded that carbohydrates are also the first materials utilized in soybean respiration.

## PATTERN OF RESPIRATION

The results of respiration measurements on the samples of seeds described in tables I and III are plotted in figures 1, 2, and 3. In these figures the curves are the best smooth curves that could be drawn from the experimental data. Each bar represents a respiration measurement; the height of the bar above the horizontal axis is equal to the average rate of respiration for the period during which the seeds were confined in the respiration flask, and

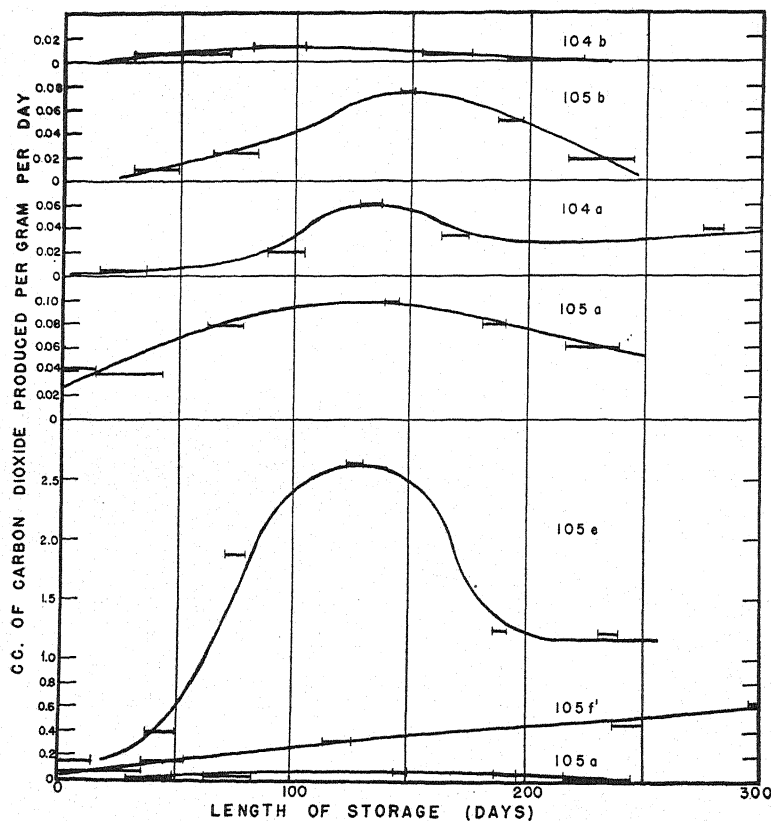


FIG. 1. The patterns of respiration of seeds in the 100 series.

the length of the bar is equal to the test interval. In order to present on one graph the respiration curves for each sample in a series, the ordinate scale in each case has been adjusted to the intensity of respiration of the sample.

Most of the curves in the above-mentioned figures exhibit a maximum rate of respiration during the storage period under investigation. It is of interest that this maximum rate is roughly proportional to moisture content. For example, in the 100 series, an increase in moisture content from 10 to 13.3 per cent. is paralleled by an increase in the maximum from 0.014 to 0.105 cc. of carbon dioxide per gram per day. At the 16.8 per cent. moisture

level, the maximum rate of respiration is 2.64 cc. of carbon dioxide per gram per day.

#### INTENSITY OF RESPIRATION

One difficulty encountered in the correlation and interpretation of the respiration data of different investigators arises from the use of an almost instantaneous respiration rate as a measure of the intensity of respiration of the seeds. Thus respiration intensity has been generally defined as the rate of production of carbon dioxide by a fixed weight of seeds kept for a stated interval from the time received at the laboratory or from the time

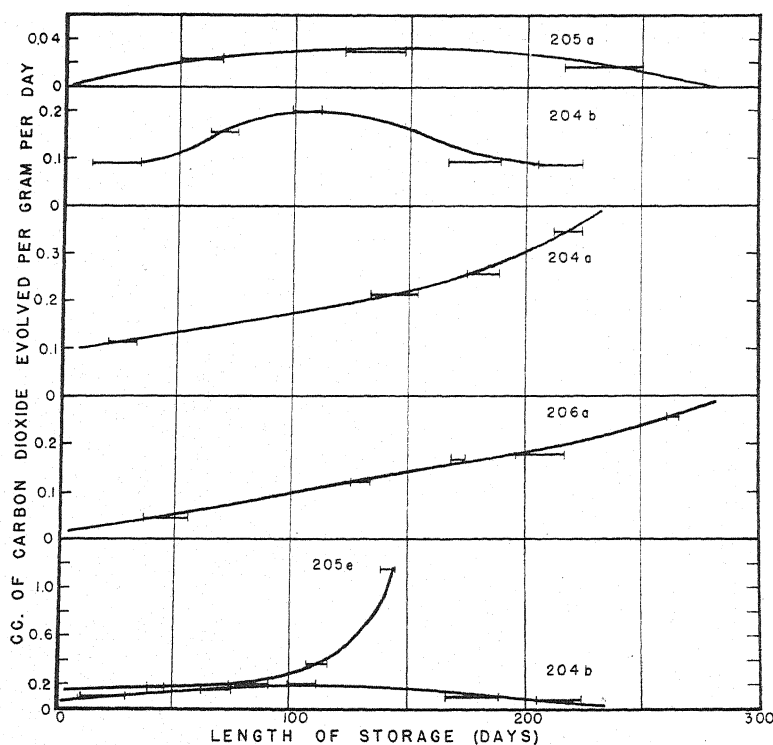


FIG. 2. The patterns of respiration of seeds in the 200 series.

conditioned to the desired moisture content. This interval may vary from a few days to years, depending on the convenience and objectives of the investigator. But such a limited definition of intensity is inadequate to provide an overall representation from the time the seeds are received until they are milled or planted, since the age and conditions of storage have a profound and often unpredictable effect on the instantaneous rate of respiration. For example, the rate of respiration measured five days after the seeds are received may have an entirely different intensity from that obtained when the rate of respiration is measured on the same seeds 30 days later. There is no *a priori* reason to prefer one measurement to the other,

and in neither case can the measured rate of respiration be properly termed the respiration intensity of the seeds.

The confusion that might be occasioned by making instantaneous rate measurements at different intervals during the storage period is illustrated by the following cottonseed respiration data: After 50 days of storage, sample 105e respired at a rate which was approximately three times as great as that of 105f' and eight times as great as that of 105a (fig. 1). After 100 days' storage, the ratio of respiration rates of the same samples was approximately 36:2.9:1. This inconsistency is even more strikingly demon-

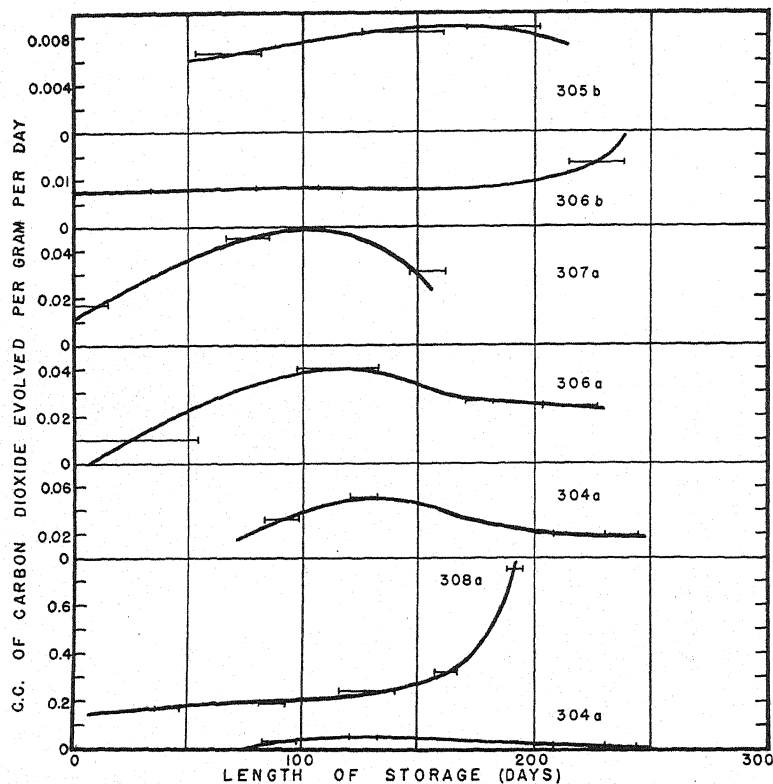


Fig. 3. The patterns of respiration of seeds in the 300 series.

strated in a comparison of the respiration rates of samples 205e and 204b (fig. 2). For the first 75 days' storage both samples had practically the same rate of respiration. It was only after this period that the curves began to diverge sharply.

Any attempt to formulate a definition of respiration must be based on the knowledge that  $I$ , the instantaneous respiration rate, is an unknown function of the time,  $t$ . For the purposes of this investigation, therefore, an averaging procedure was used and a quantity termed "average respiration intensity," has been defined by the following equation:

$$(RI) = \frac{\int_{t_0}^{t_f} I dt}{t_f - t_0} \quad (1)$$

where (RI) = average respiration intensity,

I = instantaneous respiration rate at time, t,

$t_0$  = beginning of experiment,

$t_f$  = end of experiment, and

$t_f - t_0$  = duration of experiment.

In order to evaluate (RI), periodic measurements of respiration rate were made on samples of the same seed lot over a long interval of time. All of the samples were observed over periods of not less than 100 days and generally over 200 days. The measurements so obtained formed a respiratory pattern for a given sample of cottonseed. Thus, upon integration of these patterns and division of the result by the total number of days elapsed during the experiment, an average daily respiration rate (RI) was obtained for each sample. In spite of its empirical nature, this definition, because it covers a longer period, may approach more closely than previous representations a "true" respiration intensity of resting seeds.

The value of (RI), average respiration intensity, was calculated for all of the samples illustrated in figures 1, 2, and 3, by first measuring the area under the curves by use of a planimeter. Extrapolations were avoided by

TABLE IV

VALUES FOR MAXIMUM AND AVERAGE RESPIRATION RATES OF COTTONSEED SAMPLES

SAMPLE	MOISTURE CONTENT	MOISTURE TREAT- MENT*	TOTAL RESPIRATION	STORAGE INTERVAL	AVERAGE RESPIRATION INTENSITY (RI)	MAXIMUM RATE OF RESPIRATION
	%		cc. CO <sub>2</sub> per gram	days	cc. CO <sub>2</sub> per gram per day	cc. CO <sub>2</sub> per gram per day
104b	10.0	Lowered	1.58	193	0.0082	0.014
105b	10.6	None	9.46	216	0.044	0.076
104a	12.0	None	8.16	269	0.030	0.062
105a	13.1	Raised	18.10	239	0.076	0.105
105f'	14.9	Raised	110.8	300	0.37	.....
105e	16.8	Raised	362.6	239	1.42	2.64
205a	12.5	Raised	5.44	202	0.027	0.023
204b	12.5	Lowered	29.45	212	0.14	0.20
204a	13.7	None	42.3	204	0.21	.....
206a	14.7	Raised	32.8	230	0.14	.....
205e	15.9	Raised	38.2	105	0.36	.....
305b	10.6	None	1.19	148	0.0080	0.0087
306b	10.6	None	2.09	238	0.0088	.....
307a	12.9	Raised	5.88	162	0.036	0.049
306a	13.1	Raised	6.05	226	0.027	0.040
304a	13.3	Raised	5.34	161	0.033	0.051
308a	15.7	None	44.6	158	0.282	.....

\* Refers to any conditioning treatment which may have been applied initially to either raise or lower the moisture content of the seed sample before it was set aside for respiration measurements.



measuring only that portion of the area that was in the interval between the beginning of the first and the end of the last experimental bar in each respiration pattern. By dividing the volume of carbon dioxide produced by the number of days elapsed during the experimental interval, the value of (RI) was obtained.

The results of these calculations are given in table IV together with values for the maximum respiration rates of those samples which exhibited maxima and a description of the moisture conditioning treatment employed before the seed samples were set aside for the respiration studies.

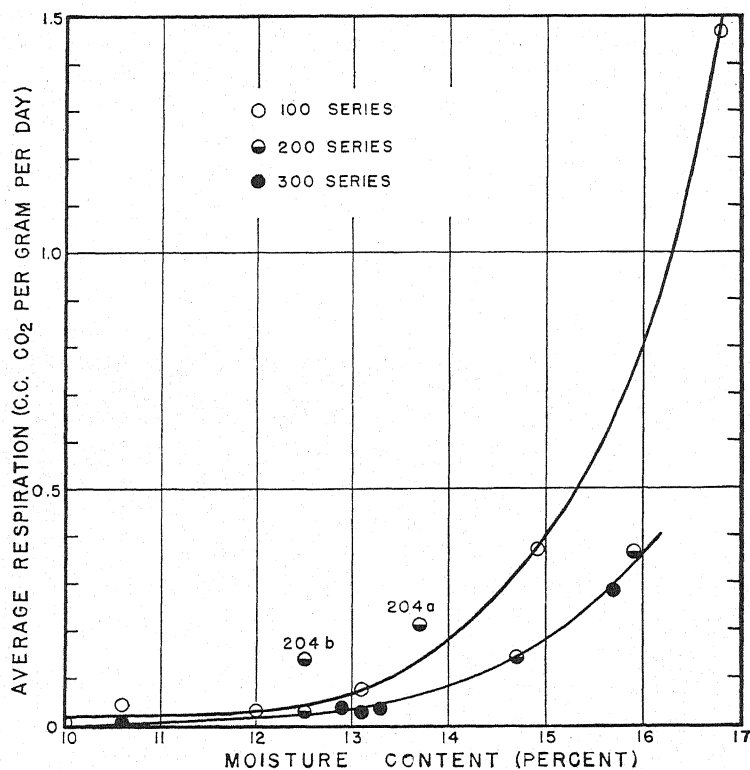


FIG. 4. The effect of moisture content on the intensity of respiration of cottonseed.

The average respiration intensity of cottonseed as a function of the moisture content of the seeds is plotted in figure 4. It is evident that the values for (RI) in any one series of seeds are smooth functions of the moisture content. It is to be expected that the vigor of the seeds and the general state of their enzymatic activity will differ for different samples of cottonseed, since differences in the conditions of growth and maturation may greatly affect the biological activity of the seeds. It is not surprising, therefore, that the seeds in one series (fig. 4) were found to respire at a different level from those in the other two series.

The difference in the behavior of the seeds in the 100 series and in the

200-300 series cannot be attributed to any significant difference in the weight of the kernels. An examination of representative samples of all three series gave values for the kernels as 56.9, 54.6, and 56.2 per cent. for the 100, 200, and 300 series, respectively.

It is apparent that once the seeds have matured, any additional time that they may spend in the boll before being harvested and ginned has no effect on their average respiration intensity. This fact is particularly clear in the results upon the 300 series where seeds of various ages are seen to fall on the same average respiration-intensity curve. It is also evident that there is no intrinsic difference between the respiration of the seeds that experienced an artificial addition of moisture and those in which there was a natural development of moisture.

The two samples, 204a, and 204b, which fell on neither of the two curves in figure 4, exhibited relatively high average respiration intensities. Both of these samples were from bolls that had been prematurely cracked open and had high moisture and free fatty acid contents when received (table I). Sample 204 was apparently the only lot that consisted of seeds that had not completely matured before being picked, a fact reflected in its respiration behavior. Measurements which will be reported in subsequent papers of this series demonstrate that this seed sample differed from the others in still other biological properties. Even though the seeds of samples 104 and 304 had likewise been obtained from prematurely cracked bolls, they, unlike those in 204a and 204b, had evidently already reached maturity in the bolls and, therefore, did not differ in respiration behavior from the seeds obtained from open bolls.

#### COMPARISON OF COTTONSEED WITH OTHER OILSEEDS

It is of interest to compare the respiration of cottonseed with that of other oilseeds. LARMOUR *et al.* (10) measured the daily respiration rates of flaxseed and sunflower seed samples of various moisture contents for periods up to 22 days after the seeds were conditioned to their final moisture levels. From their data, it is possible, therefore, to calculate the average

TABLE V

VALUES FOR AVERAGE RESPIRATION RATES OF FLAXSEED AND SUNFLOWER SEED  
AS CALCULATED FROM THE DATA OF LARMOUR *et al.* (10)

SAMPLE	MOISTURE CONTENT	AVERAGE RESPIRATION RATE* (RI)
	%	cc.
Flaxseed	12.8	0.023
	13.7	0.064
	16.5	0.19
Sunflower seed	11.8	0.039
	13.2	0.073
	14.0	0.15

\* Cubic centimeters of CO<sub>2</sub> per gram per day.

respiratory intensity of flaxseed and sunflower seed by the procedure described above. The results are shown in table V. A comparison of these values with those obtained for cottonseed (table IV) indicated that the flaxseed samples respired at a rate very close to that of the 200 and 300 series of cottonseed samples and the sunflower seed samples respired at a rate similar to that of the 100 series.

QUANTITATIVE EXPRESSION OF THE EFFECT OF MOISTURE ON THE  
RESPIRATION OF COTTONSEED

BAILEY (5) was able to convert the exponential type of curves which express the relationship between moisture content and respiratory rate into straight lines by the use of the equation:

$$y = ae^{bx} + c \quad (2)$$

where  $y$  is the rate of respiration,  $x$  is the moisture content, and  $a$ ,  $b$ , and  $c$  are constants characteristic of the material investigated. According to this

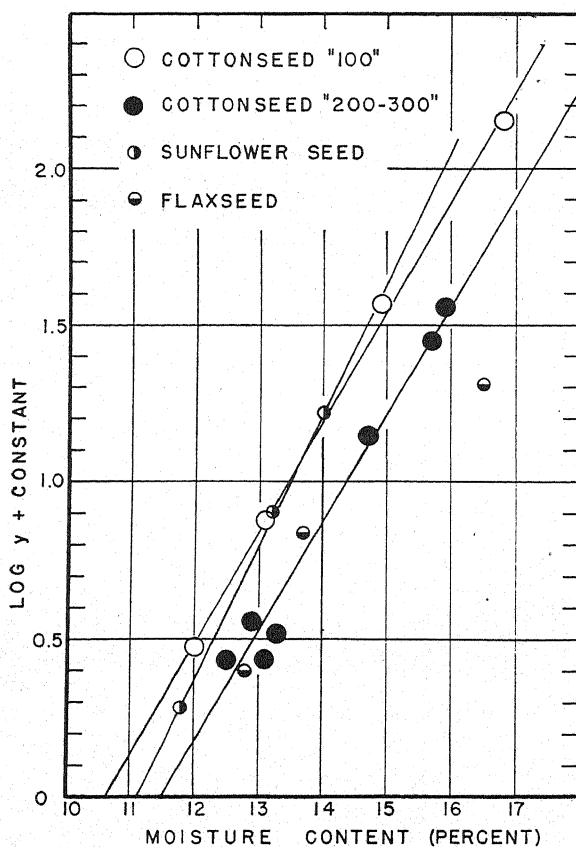


FIG. 5. Variation of the logarithm of the respiratory intensity (RI) as a function of moisture content of cottonseed, flaxseed, and sunflower seed.

equation, when  $\log (y-c)$  is plotted against the moisture content,  $x$ , a straight line is obtained. The slope of this line,  $b$ , is, therefore, a quantitative expression of the effect of moisture on the rate of respiration of seeds. Bailey was unable to use the more simple exponential relationship,

$$y = ae^{bx} \quad (3)$$

because when  $\log y$  was plotted against  $x$ , a curve was obtained instead of a straight line.

When, however, the data for the average respiration intensity (RI) of cottonseed as presented in table IV were plotted according to equation (3), a straight line was obtained for values of moisture content in the range of 12 to 17 per cent. The straight lines obtained by this method are shown in figure 5. It will be noted that the lines representing the data of the 100 and the 200-300 series have the same slope. Thus the seeds in all of the series exhibit an increase in  $\log y$  of 0.35 unit for a 1 per cent. increase in the moisture content. The only difference in the behavior of the 100 series and the 200-300 series is that the latter series is displaced 1 per cent. on the moisture axis.

Included in figure 5 are comparable average respiration intensity data for the flaxseed and sunflower seed samples of table V. The points for the sunflower seed samples fall close to those for the 100 series cottonseed samples. A straight line drawn through the points for the sunflower seed samples has a slope of  $\log y$  equal to 0.52 unit per 1 per cent. increase in moisture content. The points for the flaxseed samples do not fall on a straight line. Two of the points, however, fall very close to the straight line representing the respiration of the 200-300 series cottonseed samples. It is significant that all three types of oilseeds respire at very similar levels of intensity and that the rate of increase of the logarithm of the respiration intensity with moisture content falls within the range of 0.35 to 0.42. It would be of advantage to extend this method of analysis and observation to other types of seeds.

### Discussion

It is interesting to speculate on the nature of the relationship that exists between the moisture content of seeds and their respiration intensity. BAILEY (3) has stated that a possible explanation for the increased respiration intensity with increased moisture content is that increase in moisture serves to reduce the viscosity of the gels in the seeds and thus to increase the rate of diffusion of the cell constituents. His position is best illustrated by means of following quotation:

To restate, the production of heat is dependent upon the activity of the oxidizing enzymes of the kernel, the complex phenomenon being known as respiration. The latter is accelerated by an increase in the rate of diffusion, which in turn is dependent upon the existence of a gel, and relative viscosity of that gel. For these reasons the moisture content of sound grain determines to a considerable extent the rate of respiration and consequent liability of heating when bulk grain is stored.

In the light of newer knowledge concerning the activation and inhibition

of enzymes and of the mode of action of the enzymes within the cells, Bailey's thesis can be extended to include an explanation of the effect of moisture content on the activity of the enzymes. In dormant seeds, the food reserves remain practically unchanged during storage, even though there are present in these seeds the enzymes which are able to catalyze transformations in these substrates. In order to explain the phenomenon whereby enzyme and substrate coexist in seeds without the substrates being affected, it has been assumed that water is not distributed equally throughout the cell. There exist regions in the cell where both substrate and enzyme are in aqueous solution. These are regions of intense biological activity in which the more vigorous oxidation and hydrolytic activities take place. On the other hand, there are "islands" in the cell that are relatively water-poor. In such regions it would be expected that there would be very little oxidative or hydrolytic activity. KURSSANOV (9) has proposed that these "islands" are the seats of synthetic activity. It is highly probable that these regions are not sharply divided from each other and the change from a region of homogeneous solutions to a relative dry and non-homogeneous region is a gradual one accompanied by gradual change in the nature and degree of enzymatic activity.

In some of the first work on catalase, LOEW (11) noted that a portion of the enzyme in the tissue was not easily extractable with dilute buffers. It is well known that many enzymes are extractable from tissue only after the tissue has undergone an autolysis with the resultant disintegration of the tissue. Thus it is clear that the effective concentration of an enzyme in a cell depends not only on the total amount of enzyme present in the cell, but also on the manner in which the enzyme is bound to the tissue material. Unquestionably, much of the enzyme material in dormant seeds is rendered ineffective as a catalyst by the surrounding tissue.

Many types of enzymes are active only in the presence of reducing agents (6, 16) or other activators; *e.g.*, cyanide in papain-type enzymes (7). It is very likely, therefore, that the inactivity of some of the enzymes in dormant seeds is due also to the unavailability of activators.

On the basis of the above discussion, the effect of increased moisture content upon the respiration of seeds may be depicted provisionally as a sequence of the following events:

1. The immediate effect of increased moisture content in the cell is to increase the amount of active, readily soluble enzyme, and the amount of readily soluble substrate in the aqueous phase. This change is reflected in an increased respiratory activity within a short period after the moisture content has been raised.
2. Concomitant with the increase of the concentration of respiratory enzymes in the aqueous phase in the cell, there is also an increase of those enzymes which hydrolyze the food reserves and thus make possible the continued production of additional substrate for the oxidation reactions. Other types of hydrolytic enzymes which catalyze the disintegration of

cellular tissue are also activated, with the result that more enzymes are released into the aqueous phase.

3. The release of oxidative enzymes due to hydrolysis of the cellular tissue is reflected in an accelerated increase in respiratory activity.

4. The stimulated metabolic activity results in the formation of reducing agents and other activators which will still further increase the amount of active enzyme in the cells.

5. On the other hand, the fact must be recognized that, inasmuch as the respiration of resting seeds is not accompanied by synthetic activity, it is a very unproductive process. Instead of the energy of respiration being utilized in a furtherance of synthetic activity, most of it is released as heat. Those energy-rich compounds (such as phosphates, adenosine triphosphate, *etc.*) produced as a result of oxidation reactions, which would ordinarily be utilized in synthesis, will accumulate in resting seed and will act as inhibitors to further oxidation of the substrate.

6. Eventually a steady-state will be reached wherein the acceleration in respiratory activity due to the increase in the amount of substrate and enzymes released into the aqueous phase is balanced by inhibition due to the accumulation of products of respiration. This condition corresponds to the maxima in the respiration patterns observed in many of the cottonseed samples which were investigated.

7. Finally, the thermal destruction of enzyme activity, which takes place to greater extent in the aqueous phase than when the enzyme is adsorbed on cellular material, will result in an eventual diminution of respiratory activity.

The above description will explain the respiration patterns shown in figures 1, 2, and 3. Unquestionably, the seed lots that did not exhibit maxima in their respiration patterns would have developed them if the length of the storage interval had been sufficiently increased. Inasmuch as increase in moisture content has the effect of increasing not only the activity of existing active respiratory enzymes in the seeds, but also the rate of production of additional active enzymes, it can be understood why the respiratory intensity might be expected to be an exponential rather than a linear function of the moisture content.

At any given moisture content, the respiratory intensity will be dependent on the vigor of the seeds; *i.e.*, on the total amount of enzyme available for catalytic activity and the partition of the total enzyme between the active aqueous phase and the relatively inactive adsorbed phase. The differences between the seeds in the 100 and in the 200 and 300 series could be due either to a difference in the vigor of the seed or to a difference in the structure of the cells. Differences in cell structure could be expected to affect the equilibrium between the active and inactive forms of the enzyme.

Although in the foregoing discussion the respiration of cottonseed was attributed to enzymatic activity rather than to the activity of the micro-organisms associated with the seeds, it is true that the observed phenomena



might just as well have been explained as due to the activity of bacteria and molds. Experimental evidence is not contained in this paper to permit evaluation of the relative rôles played by micro-organisms and seed enzymes in the respiration of cottonseed. Evidence is presented in another paper of this series (2) which indicates that most of the biological activity observed in cottonseed, under the described conditions of moisture content and temperature, is due to seed enzymes.

### Summary

1. The pattern of respiration has been determined for 17 lots of cottonseed with moisture contents ranging from 10 to 17 per cent. The respiratory quotient (RQ) of resting cottonseed was found to be unity.

2. The intensity of respiration of resting seed (RI) has been defined as the average rate of respiration of the seeds over a period exceeding 100 days.

3. The average respiratory intensity of cottonseed is an exponential function of the moisture content of the seed. Mature seeds of the "Delfos" variety respired at a higher level than did the seeds of the "Coker's" or "Oklahoma Triumph" varieties.

4. The exponential-type curves which express the relationship between moisture content and respiratory intensity can be converted into linear curves by the use of the equation:

$$y = ae^{bx}$$

where  $y$  is the average respiration intensity (RI), and  $x$  is the moisture content. The value of  $b$  is 0.35 unit per 1 per cent. of moisture content for all samples of cottonseed investigated.

5. The values for the logarithm of the respiratory intensity for the "Coker's" and "Oklahoma Triumph" samples can be made to coincide with the line representing values for the "Delfos" samples by shifting them one per cent. on the moisture axis.

6. The respiratory intensity of sunflower seed samples calculated from the data of LARMOUR, *et al.* (10) can be analyzed in the same manner used for the cottonseed samples. Sunflower seed behaves in a manner very similar to the "Delfos" variety of cottonseed. The value of  $b$  for sunflower seed is 0.42 unit per 1 per cent. increase in moisture content.

7. The respiratory intensity of flaxseed samples as calculated from the data of LARMOUR, *et al.* (10) is very similar to that of the "Coker's" and "Oklahoma Triumph" varieties of cottonseed.

The authors wish to thank P. W. GULL and J. W. NEELY of the Delta Branch of Mississippi Agricultural Experiment Station, W. R. PADEN of the South Carolina Agricultural Experiment Station and H. E. DUNLAVY of the Oklahoma Agricultural Experiment Station for kindly supplying the samples used in this investigation.

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## STABILITY OF THE BUFFER SYSTEM OF LEMON JUICE<sup>1</sup>

WALTON B. SINCLAIR AND DESIRE M. ENY

(WITH FIVE FIGURES)

In previous studies (11, 12, 13), the authors have shown that the buffer systems of lemon and grapefruit juices are similar to those of pure citric acid solutions which contain amounts of free acid and salts (combined acids) in amounts equivalent to those of the juices. Consequently, the titration curves of lemon and grapefruit juices differ slightly from a pure citric acid solution provided a correction is made for the salts (combined organic acids) naturally occurring in the juices. Since these curves are nearly identical in slope, it can be reasonably assumed that the stability of the buffer system of lemon juice under various conditions is similar to that of a pure citric acid solution and its salts. To prove this hypothesis, experiments were planned to study the effects of certain factors, which as dilution, various amounts of neutral salts, sucrose and heat on the stability of the buffer properties of both lemon juice and citric acid-citrate solutions. The stability factor is measured in terms of the changes in pH of the systems under the different treatments.

Citrus juices have an efficient buffer system as shown by the fact that they resist changes in pH when hydrogen or hydroxyl ions are added to them. As citrus juices are naturally high in acidity, their maximum buffer capacity occurs on the acid side. All biological fluids are, to some degree, buffered against sudden changes in pH when strong acids or bases are added to the systems. This type of mechanism is essential for stabilizing the reaction media which are necessary for the normal function of living tissues.

### Materials and methods

The data in this investigation were obtained on pure citric acid solutions and lemon juice. A weighed amount of anhydrous citric acid was used for the solutions in which known volumes of standard NaOH were added to form the desired amount of salt with a common anion. The lemon juice samples were obtained from immature yellow lemons. Centrifuged juice was used in all experiments. The free acid content of the juices and that of the citric acid solutions were determined on aliquots by titration with phenolphthalein as indicator. The pH measurements were read on a Beckman pH meter. The combined acids of various juice samples were estimated by determining the alkalinity of the ash from an aliquot of juice. A known amount of standard HCl was added to the juice ash and the excess acid was titrated with NaOH. The alkalinity was calculated from the equivalents of HCl neutralized by the ash. This value represents closely the equivalents of combined acids present in the original sample of juice.

<sup>1</sup> Paper no. 541, University of California Citrus Experiment Station, Riverside, California.

The concentrated juice samples were prepared *in vacuo* at 50° C. The effect of heat on the buffer system of the juice was determined on samples previously boiled for 30 minutes over a free flame and then made to the original volume with distilled water. The effect of sucrose was determined by adding weighed amounts to samples of lemon juice and to citric acid solutions. In the experiments where larger amounts of sucrose were added, the samples were slightly heated over a free flame for complete solution. The neutral salt solutions of KCl, NaCl, K<sub>2</sub>SO<sub>4</sub> and KNO<sub>3</sub> were made to 1 normal. Volumetric increments of these solutions respectively were added to the lemon juice samples.

### Results

#### EFFECT OF DILUTION ON THE pH OF LEMON JUICE

According to the so-called Henderson-Hasselback equation (3) the pH

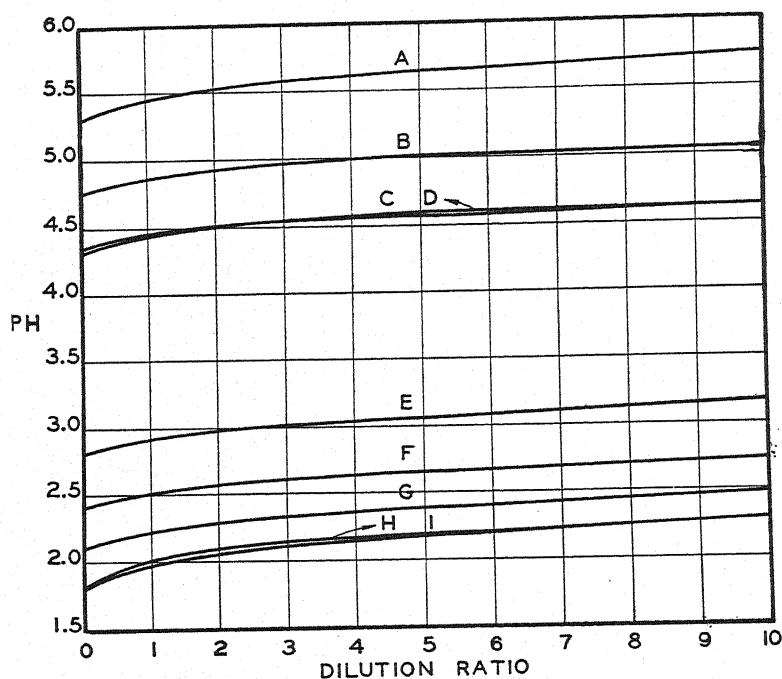


FIG. 1. Changes in pH with the dilution ratio of citric acid systems containing different amounts of salt. Curves A, 60 mg./ml. citric acid—80 per cent. salt; B, Lemon juice containing 41.92 mg./ml. of acid (as citric)—63 per cent. salt; C, Lemon juice containing 41.92 mg./ml. of acid (as citric)—50 per cent. salt; D, 60 mg./ml. citric acid—50 per cent. salt; E, Lemon juice containing 41.92 mg./ml. of acid (as citric)—13 per cent. salt; F, Lemon juice containing 41.92 mg./ml. of acid (as citric)—no salt added; G, 60 mg./ml. citric acid—3.2 per cent. salt; H, 375 mg./ml. citric acid—4.78 per cent. salt; I, Concentrated lemon juice 360 mg./ml. (as citric)—no salt added.

of solutions of weak acids and their salts are not affected to any great extent by dilution. In such systems, the dissociation constant and the ratio of the acid to salt determine the approximate pH; and, since this ratio is not

changed by dilution, a relatively small change in pH occurs. This relationship is only approximate and sufficient for most practical purposes, but it is not entirely true. In addition to the ratio of acid to salt, the total concentration of ions in the system is involved in the pH changes of a buffer solution on diluting with water. The activity coefficients change with the ionic strength. At greater ionic strengths than 0.1 normal, the difference between the individual ions in the system becomes so great as to prohibit the use of an average activity coefficient. Pure solutions of weak acids can undergo considerable dilution without any great change in pH provided the dissociation constant ( $K_a$ ) is small and the undissociated residue sufficiently high to keep  $K_a$  constant.

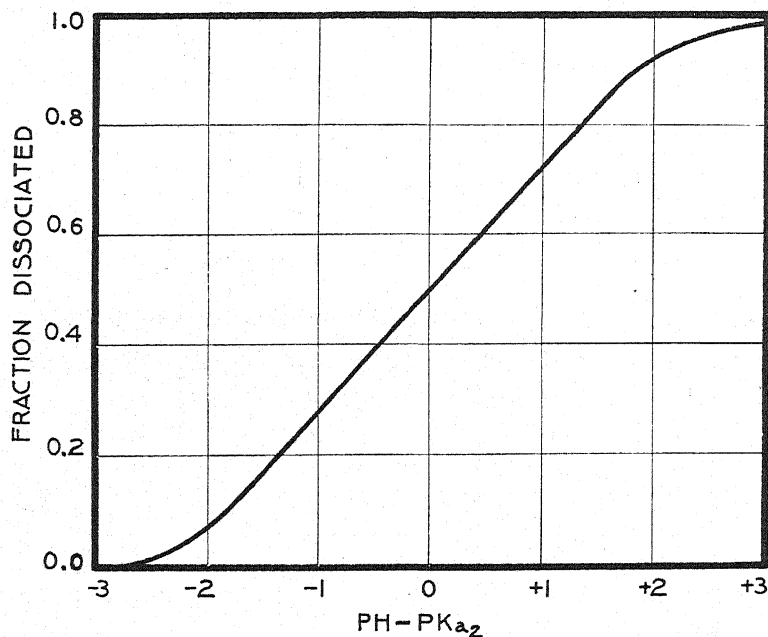


FIG. 2. The degree of dissociation of lemon juice as a function of pH.

These factors are demonstrated by the curves of figure 1 showing the relation between the pH and the dilution ratios of normal and concentrated lemon juice samples and of various citric acid solutions containing different amounts of salt. The total acid concentration and the ratio of salt to acid appear to be the important factors affecting the change in pH on dilution of lemon juices and citric acid-citrate solutions. As the difference in concentrations of the acid and salt increases in these systems, the buffer capacity is reduced and a slightly greater increase in pH occurs on dilution. A citric acid solution (curve A) containing 60 mg. per ml. citric acid and 80 per cent. salt showed a change in pH of 0.45 when diluted to ten times its volume. Another citric acid solution (curve H) containing 375 mg. per ml. and 4.78 per cent. salt showed a change in pH of 0.47 when diluted to ten times

its volume. A concentrated lemon juice (curve I) containing 360 mg. per ml. citric acid changed, under similar conditions, 0.47 of a pH. Under these experimental conditions, curves H and I are practically identical, and the changes in pH of lemon juice, when diluted or concentrated, are similar to those of a pure citric acid-citrate solution of equivalent concentrations. Although natural lemon juice has a substantial buffer capacity, this system is not at a maximum as the ratio of free acid to salt is far from unity.

Curves C and D are practically identical and they show a maximum change in pH of 0.28 when a lemon juice sample (41.92 mg. per ml. citric acid and 50 per cent. salt) and a citric acid solution (60 mg. per ml. citric

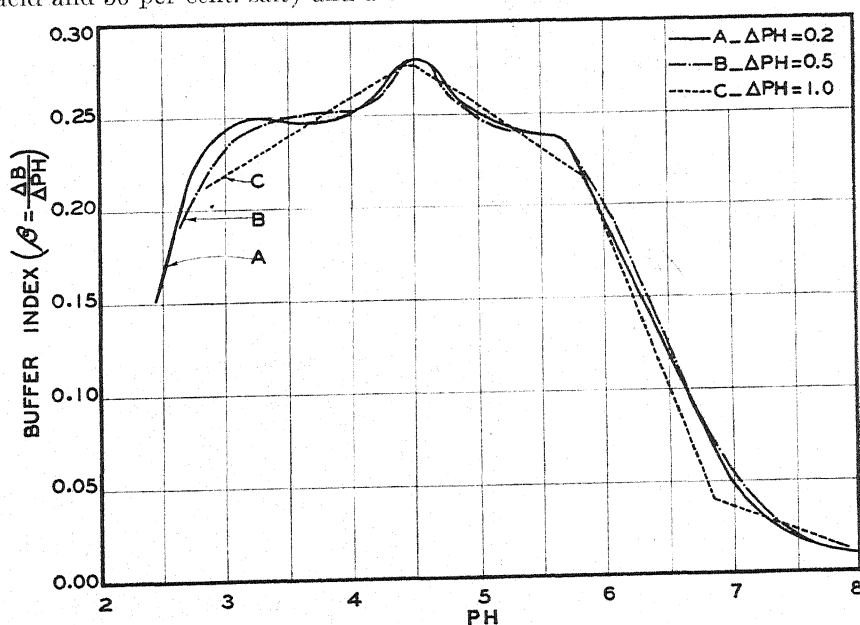


Fig. 3. Buffer index curves of a lemon juice sample expressed as a function of pH. Each curve was constructed with a different increment of  $\Delta pH$ .

acid and 50 per cent. salt) are diluted to ten times their volumes with water. In these samples, the ratio of free acid to salt is approximately equal to one; the buffer capacity is then at a maximum, and the change in pH occurring upon dilution is at a minimum. Curves B, E, F and G (fig. 1) show the variations of pH on dilution where the salt/acid ratio deviates from 1.

The effective buffer range of lemon juice on both sides of  $pK_{a2}$  is shown in figure 2. This curve is similar in shape to the fundamental curve generally ascribed to weak acids and their salts (3). When 50 per cent. of the acid of lemon juice is in the salt form (or dissociated), the pH is equal to  $pK_{a2}$ . At this point the juice has a maximum buffer capacity and the ratio of free acid to salt is unity. The buffer range of lemon juice is represented by the straight portion of the curve and extends over an approximate range of  $pH = pK_{a2} \pm 1.8$ .



## BUFFER INDEX OF LEMON JUICE

The buffer properties of lemon juice are more accurately indicated by the buffer index curves of figure 3. These curves represent the reciprocal of the slope of the titration curve (see fig. 5) when the amount of base used in the titration is expressed in equivalents. If the pH values are plotted on the abscissa, the buffer index measures the degree of slope of the titration curve. The buffer index is mathematically expressed by the differential ratio  $\frac{dB}{dpH}$ , where dB is an infinitesimal part of an equivalent of base, and dpH is an infinitesimal change in pH. The buffer index curves (fig. 3, curves B and C) calculated with larger increments of  $\Delta pH$  shows trends rather than accurate representations of the buffer system. It can be seen

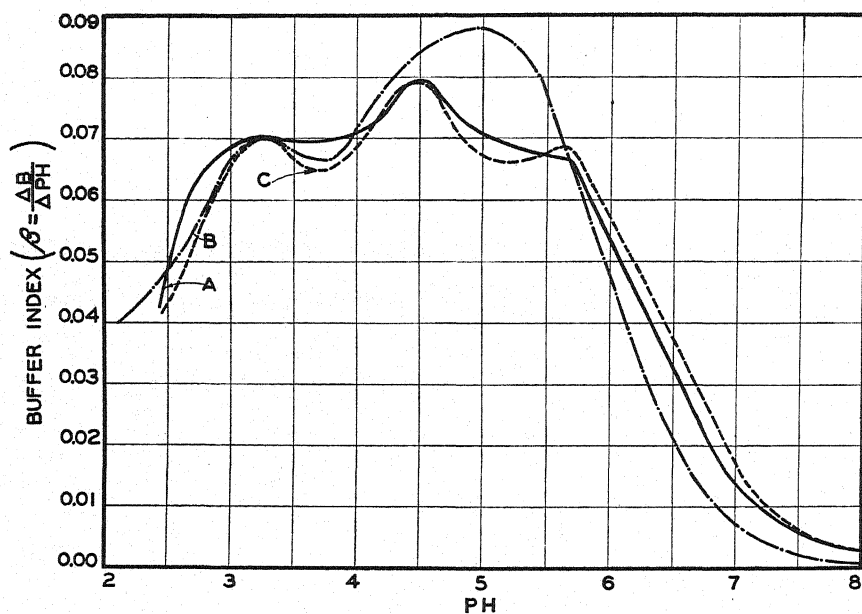


FIG. 4. The buffer index curve of lemon juice (A) plotted in terms of a 0.1 M solution, compared with the theoretical curve (B) of HASTINGS and VAN SLYKE (6) for 0.1 M citric acid solution, and with the experimental curve (C) for citric acid of 0.1 M concentration.

that the smaller the increment of change in pH ( $\Delta pH$ ) the more nearly the experimental curve approaches the real buffer index curve of lemon juice. The three curves were determined on the same sample of juice, but each one was plotted from values calculated with increments of  $\Delta pH$  of 0.2, 0.5 and 1.0, respectively. Curve A is calculated from the data using  $\Delta pH = 0.2$ . Note that the maximum buffer capacity of lemon juice is at a pH of 4.4 which is in close agreement with the dissociation exponent of the second dissociation constant of citric acid ( $pK_{a2} = 4.39-4.50$ ). Curve A also shows in detail that the buffer capacity of lemon juice is between pH 2.7 and 5.6. The two minor inflections indicate the position of  $pK_{a1}$  and  $pK_{a3}$ .

The position of the curves on the graph, with respect to the buffer index values, is influenced by the concentration of the free acid in the lemon juice samples. In other words, the buffer capacity of a solution is governed by the concentration of the components. As the buffer index cannot be represented by a constant, it must be defined in terms of a given pH. This particular lemon juice sample had a total acidity of 68.16 mg. per ml. of citric acid and citrate. This is equivalent to a 0.355 molar solution in terms of the total citric acid radicle in the sample. The maximum buffer index ( $\beta$ ) of the lemon juice was 0.279 at pH 4.4. By simple calculation from these data, the maximum molar buffer index ( $\beta_M$ ) of lemon juice at pH 4.4 would be 0.79. According to VAN SLYKE (14), citric acid has a maximum  $\beta_M$  of 0.84. The  $\beta_M$  of lemon juice agrees surprisingly well with that of pure citric acid solutions.

The agreement of the  $\beta$  values for lemon juice with those of a citric acid solution are further illustrated in figure 4. The buffer index values for lemon juice (curve A) are plotted in terms of 0.1 M concentration. This was done so a comparison could be made with the theoretical curve of HASTINGS and VAN SLYKE (6) for 0.1 M citrate solutions (curve B). Their curve was estimated and constructed by graphic summation from the three pKa values of citric acid. The distinct difference between these two curves is in the position of the maximum buffer index which is at pH 5.0 for the theoretical curve and at pH 4.4 for the lemon juice. On the contrary, the experimental curve obtained for 0.1 M solution of citric acid (curve C) agrees closely with curve A for lemon juice.

#### EFFECTS OF NEUTRAL SALTS ON THE pH OF LEMON JUICE

Although most biological systems are buffered, the reactions occurring in the tissues are carried out in relatively high concentrations of mixed electrolytes. In plant tissue fluids, changes occurring in pH are not accompanied by corresponding changes in total salt concentration. In lemon juice and other citrus fruits, the organic acids are slightly ionized, and the inorganic salts that affect the pH are those that liberate free cations during the metabolism of the fruit. One important characteristic of the buffer systems of citrus juices is that their total salt or ionic concentration can be reduced by dilution, and at the same time, the system maintains an adequate buffer intensity. Cells of plant tissues, in general, can become hydrated, thus decreasing the effect of relatively high concentrations of electrolytes, and maintain a constant reaction medium with respect to changes in hydrogen-ion concentration.

The data in table I show the effect of different concentrations of neutral salts on the pH of lemon juice. In concentrations as high as 10 m.e. per ml. of lemon juice, KCl and NaCl produced no significant change in pH. With the same concentrations of  $K_2SO_4$  or  $KNO_3$ , increases in pH of 0.18 and 0.19, respectively, were recorded. Although no reasons are given for these results, HAYNES and BROWN (7) report a similar effect of  $K_2SO_4$  on apple

TABLE I

EFFECT OF NEUTRAL SALTS ON LEMON JUICE

NEUTRAL SALTS ADDED TO JUICE	pH OF LEMON JUICE (43.68 MG./ML. FREE ACID (AS CITRIC)) WITH VARIOUS AMOUNTS OF NEUTRAL SALTS			
M.E./ML.	KCl	K <sub>2</sub> SO <sub>4</sub>	NaCl	KNO <sub>3</sub>
0.0	2.32	2.32	2.32	2.32
0.68	2.32	2.32	2.32	2.32
1.37	2.32	2.33	2.32	2.32
2.05	2.31	2.34	2.31	2.32
2.73	2.31	2.35	2.31	2.33
3.41	2.31	2.37	2.31	2.34
4.10	2.31	2.40	2.31	2.35
4.70	2.31	2.40	2.31	2.35
5.46	2.31	2.42	2.32	2.37
6.14	2.31	2.44	2.32	2.41
6.83	2.31	2.46	2.32	2.43
8.00	2.31	2.48	2.32	2.47
9.00	2.32	2.49	2.33	2.49
10.00	2.32	2.50	2.33	2.51

juice. If either the cations or anions of the neutral salts had reacted chemically with some substance in the lemon juice, a shift in pH of the sample would have occurred. The different neutral salts produced no marked effect on the pH of lemon juice. This is evidence that the neutral salts remained in ionic form. It is generally recognized that salts of strong acids and strong bases are neutral in aqueous solutions. As 85 per cent. of the total ash of the edible portion of the lemon is soluble in the juice, it is highly probable that most of the ash constituents exist in the ionic state. The cation-anion balance of the neutral salts does not influence the alkaline ash and pH of the juice. It is the excess cations that are available for salt formation with the organic acids which affect the pH.

#### EFFECT OF SUCROSE ON BUFFERED SOLUTIONS

The soluble solids of citrus fruits are chiefly composed of soluble sugars and organic acids. In biological systems, the sugars play an important rôle in the osmotic activity of the cells, but produce no appreciable effect on the pH. The dissociation constants of the sugars are of such magnitude (of the order of  $10^{-13}$ ) that they are probably without buffer effect in citrus juices. In table II are recorded the pH values of different buffered solutions containing various amounts of sucrose. The first two samples are lemon juices, and the others are citric acid solutions buffered at different pH values by varying the salt content. No significant change in pH occurred in any of these buffered systems that contained a concentration of sucrose as high as 50 per cent. As sucrose forms a hexahydrate in water (9), one mole of sucrose dissolved in 1000 grams of water would combine with six moles of water, leaving 892 grams of water for solvent purposes ( $1000 - (18 \times 6) = 892$  grams). In the presence of lyophilic colloids the amount of water taken up by sucrose varies (5). The small amount of lyophilic colloids present in

TABLE II  
EFFECT OF SUCROSE ON LEMON JUICE AND BUFFERED SOLUTIONS

TYPE OF BUFFER SOLUTION	PH OF VARIOUS SOLUTIONS CONTAINING DIFFERENT PERCENTAGES OF SUCROSE						
	0	5	10	15	20	25	50
Fresh lemon juice—33.34 mg./ml. free acid (as citric) .....	2.43	2.43	2.43	2.43	2.43	2.43	2.43
Concentrated lemon juice diluted 1 to 5—60.16 mg./ml. free acid (as citric) .....	2.18	2.18	2.18	2.18	2.18	2.18	2.18
Citric acid—60 mg./ml. + 3.2% salt* .....	2.12	2.12	2.12	2.12	2.12	2.12	2.12
Citric acid—60 mg./ml. + 6% salt .....	2.40	2.40	2.40	2.40	2.40	2.40	2.39
Citric acid—60 mg./ml. + 80% salt .....	5.34	5.34	5.34	5.34	5.34	5.34	5.32
Citric acid—60 mg./ml. + 50% salt .....	4.33	4.33	4.33	4.33	4.32	4.32	4.32
Citric acid—360 mg./ml. + 3.1% salt .....	1.70	1.70	1.70	1.70	1.70	1.70	1.69
Citric acid—360 mg./ml. + 35% salt .....	3.40	3.40	3.40	3.40	3.40	3.41	3.40

\* A quantitative amount of NaOH was added to obtain the desired percentage of salt.

centrifuged lemon juice indicates that the amount of water available for solvent purposes would not be affected. Under these experimental conditions, the only means by which sucrose could produce a change in pH of lemon juice is by reducing the apparent volume of the solvent of the system. By so doing, the acid and salt constituents that affect the pH of the juice would be increased accordingly. The ratio of free acid to salt, however, would not be changed. The amount of water used to form the hexahydrate in a 50 per cent., by weight, sucrose solution would be approximately 15 per cent. Such a small reduction in volume would hardly cause a significant change in pH.

#### EFFECT OF HEAT ON THE BUFFER CAPACITY OF LEMON JUICE

Citric and malic acids in aqueous solutions are chemically stable at boiling temperatures. Since the buffer capacity of citrus juices is due chiefly to these nonvolatile organic acids and their salts (10, 11), it is reasonable to presume that the pH of these juices should not undergo any great change at the boiling point and at atmospheric pressure. BARTHOLOMEW and SINCLAIR (2) have already shown that the course of the titration curve for orange juice that was boiled for 45 minutes was similar to that for normal juice. The slightly higher pH value of the boiled juice showed that this juice required slightly less NaOH than unboiled juice for neutralization. The titration curves of juice samples, boiled for 5, 10, 15 and 30 minutes, respectively, were so close to those of normal juice that it was impractical to put them on the same graph. As the ratio of the free acid to salt is much greater in the lemon than in the orange, it was decided to compare the titration curves of boiled and normal lemon juice for the purpose of measuring the effect of heat on the buffer capacity of this system. This type of experiment should show whether the heat decomposed or volatilized any of the acid constituents and thus lessened the amounts of NaOH required for

neutralization. It should show, also, the effect of heat on the soluble pectins or on any other constituents that might hydrolyze and thus increase the amounts of NaOH required for neutralization.

The effect of heat on the buffer system of lemon juice is amply demonstrated by the titration curves for normal lemon juice (fig. 5, curve A) and for a portion of the same juice boiled for 30 minutes (fig. 5, curve B). For comparative purposes, a titration curve is drawn of pure citric acid with a concentration of free acid equivalent to that of lemon juice at its natural pH. The course of the titration curve of lemon juice is not affected

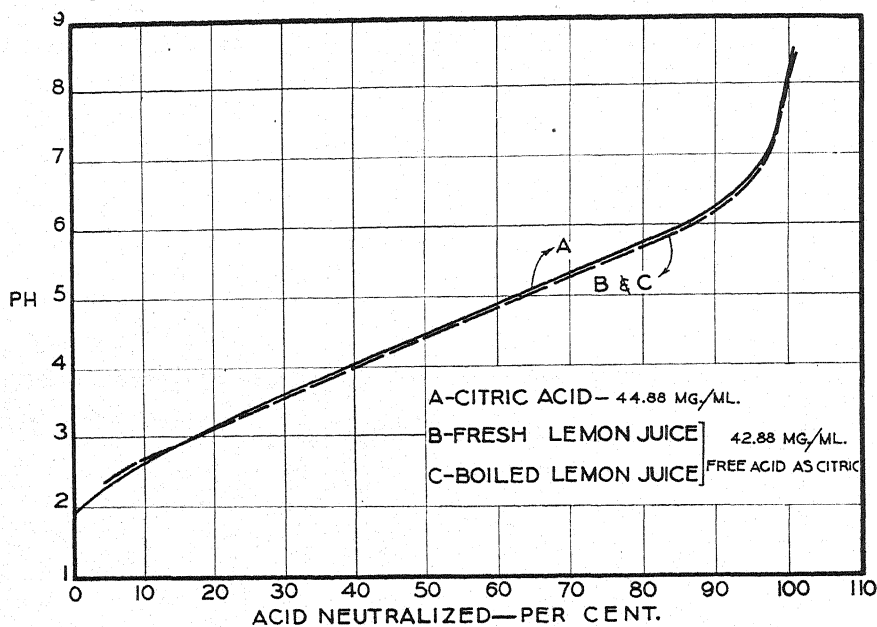


FIG. 5. Titration curves of lemon juice normal and boiled and pure citric acid solution. One curve was drawn for B and C as their values were too close to separate graphically. A correction was made on the curves B and C for the salt naturally occurring in the juice.

by heat. There is no significant difference in these three curves at any given pH between 0 and 100 per cent. neutralization. These results agree with those of DUNNE (4) who showed that boiled and filtered sap of wheat and buckwheat plants gave titration curves identical with those of the fresh sap; of BARNES (1) who observed that the hydrogen-ion concentration was only slightly lower in cooked apple juice than in fresh; and of MARSH (8) who showed that the buffer capacity of juice of nonacid vegetables was affected only slightly when heated in acid solution.

### Summary

The buffer system of lemon juice is similar to that of citric acid-citrate solutions of equivalent concentrations. In lemon juice samples and pure

citric acid solutions, the increase in pH with the dilution ratios is influenced by the concentrations of total acid and salt. The smallest change in pH on dilution occurred in systems where the ratio of free acid to salt was unity. As lemon juice contains a large ratio of free acid to salt, the changes in pH are relatively large with increase or decrease in juice volume. The increase in pH with dilution is approximately the same for lemon juice and pure citric acid solutions of equivalent concentrations. The efficient buffer capacity of lemon juice extends over an approximate range of  $\text{pH} = \text{pK}_{a_2} \pm 1.8$ . This agrees with the maximum range of the buffer index curve. The buffer index value of lemon juice is at a maximum at pH 4.4. This value is in agreement with the  $\text{pK}_{a_2}$  of a pure citric acid solution. The two minor inflections on the curve indicate  $\text{pK}_{a_1}$  and  $\text{pK}_{a_3}$  values of lemon juice. In concentrations as high as 10 m.e. per ml. of lemon juice, KCl and NaCl produced no significant change in pH. With the same concentrations of  $\text{K}_2\text{SO}_4$  and  $\text{KNO}_3$ , increases in pH of 0.18 and 0.19, respectively, were recorded.

In concentrations as high as 50 per cent. by weight, sucrose did not change the pH values of various lemon juices and citric acid solutions which had different ratios of free acid to salt.

That the buffer capacity of lemon juice is not affected by heat is demonstrated by the fact that boiled and normal juices have identical titration curves.

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## CHANGES IN THE COMPOSITION OF SQUASH DURING STORAGE<sup>1</sup>

THOMAS G. PHILLIPS

Studies by LE CLERC DU SABLON in 1905 (5) established the general nature of the changes occurring in the fruits of Cucurbits during maturation and storage. At maturity the solids of the fruits of *Cucurbita pepo* var. *courge olive* were high in starch and relatively low in sugar. During storage the conversion of starch to sugar was rapid, as was also the loss of sugar by respiration. On long storage much of the total carbohydrate had disappeared and the water content of the fruit had increased markedly.

Later reports have added detail to these findings and have served to show the great variations in composition among the many varieties of the species commonly called pumpkin or squash. This work has been reviewed and greatly extended in the recent paper by CULPEPPER and MOON (2).

Members of the staff of the New Hampshire Agricultural Experiment Station, under the leadership of the Department of Horticulture, have been engaged for some years in a study of the problems connected with the storage of squash. During three seasons samples of three varieties of squash have been taken at harvest and after various periods of storage. It was expected that the chemical analysis of these samples would furnish more detail, and especially more exactly quantitative information, about changes during storage than has been available.

### Material and methods

The varieties studied were Blue Hubbard and Buttercup, strains of *Cucurbita maxima*, and Butternut, a strain of *C. moschata*. The fresh weight of each sample was 150 gm., 30 gm. of which were taken from the edible portion of each of five fruits selected at random from a large lot of apparently uniform specimens. The samples were preserved in 80 per cent. alcohol and extracted and analyzed by essentially the same methods used in earlier studies of the timothy plant (6).

As preliminary tests showed that lignin is either absent or present in very small amounts, this determination was omitted. Total pectin was determined by a modification of the method used by DOTY *et al.* (3) as follows: A sample of the residue from the alcohol extraction of sufficient size (0.0625 to 0.2500 gm.) to yield not more than 0.0250 gm. of calcium pectate was extracted for four successive 30-minute periods with 40 ml. of boiling N/30 HCL. The filtrate and washings from each extraction were neutralized immediately to methyl red indicator. The combined extracts were diluted to 300 ml. in a 600-ml. beaker; 100 ml. of 0.1 N NaOH were added, and the solution was let stand overnight. It was then acidified with 50 ml. N CH<sub>3</sub>COOH, after which 50 ml. M CaCl<sub>2</sub> were added. After allowing to

<sup>1</sup> Published with the permission of the Director as scientific contribution no. 105 of the New Hampshire Agricultural Experiment Station.

stand until the flocculent precipitate had settled, much of the supernatant liquid was siphoned off, leaving the precipitate suspended in a volume of 100-150 ml. To this, 0.1500 gm. of dry asbestos was added, the mixture was boiled for a few moments, then filtered on an asbestos pad weighed in a Gooch crucible, and washed thoroughly with hot water, then with alcohol and finally with ether. Crucible and contents were dried in an oven at 102° C. and weighed. Duplicate portions of the asbestos, 0.1500 gm., were carried through the boiling, filtration, washing and drying to serve as blanks.

In the earlier samples starch was determined as the fermentable sugars produced from the alcohol extracted residue by hydrolysis with  $N H_2SO_4$  for 2.5 hours, and also by the action of Taka diastase for 40 hours. The two methods agreed so closely that only acid hydrolysis was used with the later samples.

The absence of maltose from four of the samples of Blue Hubbard taken at various periods from harvest to six months storage was established by the following procedure. In one portion of the cleared extract total sugars were determined by the reducing power after treatment with invertase. In another portion the action of invertase was preceded by the same Taka diastase treatment that is used for starch. As the diastase treatment hydrolyzes maltose completely, the reducing power of the sample should be increased by this treatment if maltose were present. No increase was found in the squash samples. The same treatment, applied to the extract from the endosperm of germinating corn, indicated large amounts of maltose. The method appears conclusive as far as proving the absence of maltose is concerned. Studies of the use of this method for the quantitative determination of maltose in the presence of other sugars have not as yet been completed.

In sampling the 1943 harvest the loss in fresh weight of each fruit was determined from harvest until the time of sampling. These figures, with the analysis of the samples, made possible an estimate of the total change in amount of the various constituents during the storage period. A partial statement of these results has been reported in a general bulletin on storage problems (7). A more elaborate system was adopted for the 1944 harvest. About 35 fruits of each of the three varieties were kept in a separate storage room. The weight of each squash was recorded at regular intervals until it was used as a sample or until it spoiled. With the Blue Hubbard this was continued for six months. All the others had been lost before that time.

On sampling, the inner fiber and seeds were separated from each fruit. Their fresh weight was determined, and the parts from the five fruits were mixed and sampled. After extraction with 80 per cent. alcohol the seed samples were extracted with ether, and the ether-soluble materials in the alcohol extract were determined. The sum of these two items is listed as "ether extract" in the presentation of the results. Pectin appeared to be practically absent from the seed. The fiber samples were analyzed by the same methods used for the flesh.

### Results and discussion

The composition of the edible portion of the squash is presented in table I. The figures are the averages of the samples taken at harvest and at various storage periods during three seasons. The amounts listed under "Total carbohydrates" are the sums of those for glucose, fructose, sucrose and starch, all calculated to their starch equivalent.

The results of earlier studies are confirmed in the following respects: (1) The rapid disappearance of starch; (2) the increase in sugars, at least to three months' storage; (3) the decrease in total carbohydrates; and (4) the increase in water content. It appears that the water content depends on the balance between the loss of water by evaporation and its gain by respiration. The Blue Hubbard lost fresh weight more slowly than did the other varieties. Its water content increased markedly, that of the Buttercup only slightly, and that of the Butternut not at all.

In a chemical study of the development of fruits of cucurbits ARASIMOVICH (1) reported glucose always in excess of fructose. In the present study fructose was found in excess of glucose in all samples taken at harvest. The proportion of glucose increased during storage. Table I indicates that Butternut is an exception in this respect, but in a single fruit which had been in storage for a year, glucose was 5.52 per cent. and fructose 3.25 per cent. of the dry weight.

This trend toward an increased proportion of glucose is opposite that found in haplocorms of timothy (4) in which carbohydrate is stored as fructosan. In that case it was suggested that relatively slow interconversion of glucose and fructose might explain the accumulation of fructose. The same statement could explain the accumulation of glucose in the present case, since the reserve carbohydrate is starch. The carbohydrate relationships are complicated by rapid respiration. At present there is, in this case, no direct evidence as to which sugar is used most rapidly in this process.

The relatively large amount of sucrose in Butternut after storage is striking. This may be one factor in the very desirable flavor of this variety. Since no maltose was found in such samples of Blue Hubbard as were tested for it, the participation of amylases in the conversion of starch to sugar seems doubtful. Phosphorylation would appear to be more probable. In percentage of dry weight, both cellulose and pectin increase during storage. Since total nitrogen increases also, it is possible that all these increases are relative rather than absolute.

The composition of the fiber differs in detail, but not strikingly, from that of the edible portion. During storage its changes in composition differ from those of the flesh, especially in the following respects: (1) There is an appreciable loss of water; (2) the loss of total carbohydrates is much more rapid; and (3) the gains in cellulose and pectin are much more pronounced.

The water content of the seeds is relatively high at harvest and decreases greatly during storage (table II). Total carbohydrates are low compared with other parts of the fruit, but they are considerably higher in the seeds

TABLE I  
AVERAGE COMPOSITION IN PERCENTAGE DRY WEIGHT OF THE EDIBLE PORTION OF SQUASH AT  
VARIOUS STORAGE PERIODS, 1942-44

	BLUE HUBBARD				BUTTERCUP			BUTTERNUT		
	HARVEST	2 WEEKS	3 MONTHS	6 MONTHS	HARVEST	2 WEEKS	3 MONTHS	HARVEST	2 WEEKS	3 MONTHS
	%	%	%	%	%	%	%	%	%	%
Soluble solids .....	35.05	46.82	71.82	71.71	20.89	30.90	55.38	24.96	36.82	58.18
Glucose .....	9.71	14.53	25.30	30.04	3.52	7.34	16.85	2.18	2.68	0.63
Fructose .....	10.94	14.06	20.56	15.60	4.77	7.50	12.04	4.72	5.08	3.08
Total reducing sugars .....	20.65	28.59	45.86	45.64	8.29	14.84	28.89	6.90	7.76	3.71
Sucrose .....	3.56	5.07	10.84	6.65	4.43	7.46	14.42	8.07	18.23	39.84
Total sugars .....	24.21	33.66	56.70	52.29	12.72	22.30	43.31	14.97	25.99	43.55
Starch .....	42.32	28.54	4.41	0.48	53.44	43.32	14.54	52.86	40.57	18.48
Total carbohydrates as starch .....	64.01	58.96	55.76	47.68	64.36	63.29	54.16	66.94	64.43	59.41
Cellulose .....	5.82	7.52	9.50	13.15	5.59	6.50	10.27	6.43	6.95	8.02
Pectin .....	3.72	4.28	5.88	6.95	3.26	3.33	6.28	3.84	3.62	5.38
Total N as protein .....	6.14	6.08	6.26	8.17	5.22	5.35	7.09	6.54	6.94	7.80
Solids % F.W. ....	14.43	13.42	13.01	10.60	24.31	24.51	18.49	17.59	17.34	17.56

TABLE II  
COMPOSITION OF THE SEED OF SQUASH AT VARIOUS STORAGE PERIODS IN PERCENTAGE DRY WEIGHT

	BLUE HUBBARD				BUTTERCUP			BUTTERNUT		
	HARVEST	2 WEEKS	3 MONTHS	6 MONTHS	HARVEST	2 WEEKS	3 MONTHS	HARVEST	2 WEEKS	3 MONTHS
Total carbohydrates as starch	%	%	%	%	%	%	%	%	%	%
Cellulose	15.57	5.26	3.65	4.62	9.94	5.66	6.75	26.10	12.84	10.76
Ether extract	14.96	11.64	13.94	12.46	10.76	11.11	11.87	8.34	8.61	9.09
Total N as protein	12.92	34.87	29.91	31.56	29.05	35.08	31.92	8.47	30.54	28.74
Solids % F.W.	23.21	28.99	28.72	31.29	27.26	29.72	28.39	21.09	29.40	31.16
	23.48	48.64	58.84	61.28	34.12	54.76	67.28	29.40	46.60	66.12



TABLE III  
CONSTITUENTS IN GRAMS PER KILOGRAM FRESH WEIGHT AT HARVEST. AVERAGES, 1942-44

	BLUE HUBBARD				BUTTERCUP			BUTTERNUT		
	HARVEST	2 WEEKS	3 MONTHS	6 MONTHS	HARVEST	2 WEEKS	3 MONTHS	HARVEST	2 WEEKS	3 MONTHS
Solids .....	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Soluble solids .....	144.3	129.9	117.5	89.0	243.1	221.1	143.0	175.9	154.1	131.6
Glucose .....	50.58	60.82	84.39	63.82	50.78	68.32	79.19	43.90	56.74	76.56
Fructose .....	14.01	18.87	29.73	26.74	8.56	16.23	24.10	3.83	4.13	0.83
Total reducing sugars .....	15.79	18.26	24.16	13.88	11.60	16.58	17.22	8.30	7.83	4.05
Sucrose .....	29.80	37.13	53.89	40.62	20.16	32.81	41.32	12.13	11.96	4.88
Total sugars .....	5.14	6.59	12.74	5.92	10.77	16.49	20.62	14.20	28.09	52.43
Starch .....	34.94	43.72	66.63	46.54	30.93	49.30	61.94	26.33	40.05	57.31
Total carbohydrates as starch .....	61.07	37.07	5.18	0.43	129.91	95.78	20.79	92.98	62.52	24.32
Cellulose .....	92.37	76.59	65.52	42.44	156.46	139.93	77.45	117.75	99.29	78.18
Pectin .....	8.40	9.77	11.16	11.70	13.59	14.37	14.69	11.31	10.71	10.55
Total N as protein .....	5.37	5.56	6.91	6.19	7.93	7.36	8.98	6.75	5.58	7.08
	8.86	7.90	7.36	7.27	12.69	11.83	10.14	11.50	10.69	10.26

of the Butternut than in those of the other varieties. The relative increases in ether extract and in total nitrogen are striking. At harvest the seeds of the Buttercup variety appeared more mature than the others, and the changes listed above are less pronounced.

In assembling the results of the 1944 samplings it became evident that extreme variations occurred in two respects: the moisture content of the fruits, and the proportion of fiber and seed in them. Individual determinations of total solids were made on each of the five fruits used for one of the Blue Hubbard samples. The results were: 15.76, 11.16, 10.84, 11.91 and 14.36 per cent. The percentages of the total solids of the whole fruit present in fiber and seeds were also extremely variable.

When the samples for this season were taken it was hoped that the results

TABLE IV

LOSSES DURING STORAGE IN PERCENTAGE OF THE AMOUNTS PRESENT AT HARVEST

	2 WEEKS	3 MONTHS	6 MONTHS
	%	%	%
Blue Hubbard			
Fresh weight .....	3.19	9.72	16.06
Solids .....	9.98	18.57	38.32
Total carbohydrates .....	17.08	29.07	54.05
Buttercup			
Fresh weight .....	9.79	22.65	.....
Solids .....	9.05	41.18	.....
Total carbohydrates .....	10.56	50.50	.....
Butternut			
Fresh weight .....	11.15	25.05	.....
Solids .....	12.39	25.18	.....
Total carbohydrates .....	15.68	33.60	.....

of their analysis might make possible the calculation of the material changes in flesh, fiber and seed during the storage period. The variations recorded above show that these calculations from a single season's sampling would have little, if any, value. For this reason such results for fiber and seed are not recorded. They do, however, provide some indication that there is little, if any, transfer of material from flesh to fiber and seed during storage.

A more reliable estimate of the material changes in the edible portion of the fruits was obtained as follows: Fiber and seed were omitted from the calculation. Together they comprise a comparatively small part of the whole, and as there appears to be no great change in their total weight during storage, the error introduced in this way is small. The figures in table I were used for the composition at the various storage periods. Since these are the averages for three seasons, they are more representative than the results for any one season. Since the loss in fresh weight of a large number of fruits was determined during the 1944 season, the averages of these losses, which are presented as part of table IV, were used in the calculation. The estimates obtained in this way, of the material changes in the edible portion

corresponding to a given fresh weight of fruit at harvest, are presented in table III.

In spite of the sampling difficulties, the major changes are quite evident. These include pronounced losses in solids, in starch and in total carbohydrates. The results indicate in most cases some increase in cellulose and pectin and some loss in total nitrogen. It is doubtful if the samples are sufficiently representative to provide conclusive evidence on these points. Both tables I and III emphasize the large amounts of sugars present, much of which might be lost by certain methods of processing. From the practical point of view the losses of fresh weight, of total solids and of total carbohydrates are especially important. These losses are presented in table IV in percentage of the amounts present at harvest.

### Summary

A study of the composition of samples of three varieties of squash taken at harvest and after various periods of storage during three seasons leads to the following conclusions:

1. The conversion of starch to sugar is rapid and becomes practically complete on long storage.
2. The loss of carbohydrates is rapid, amounting to about one-third of the amount present at harvest after three months' storage, and one-half or more after six months' storage.
3. The proportion of glucose in the total sugars increases during storage.
4. In the Butternut, the proportion of sucrose in the total sugars is very high.
5. Since maltose was absent from the samples tested for it, it is doubtful if amylases are concerned in the transformation of starch.
6. The results indicate slight losses in protein and slight gains in cellulose and pectin. It is doubtful, however, that the sampling was adequate to provide conclusive evidence on changes in cellulose and pectin content.

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## POLAR DISTRIBUTION OF RESPIRATORY RATE IN THE ONION ROOT TIP

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### Introduction

Since the root tip of *Allium cepa* is being used more and more in physiological investigations, it was considered of value to investigate and compare the respiratory rate of different regions in this polar tissue. LUND and KENYON (8) observed that the region of active mitotic division in the root tip had the greater rate of reduction of methylene blue when compared to more basal regions and that this apical region was electropositive to the higher regions of the root. They also found that there was the same polar distribution in the production of acid ( $\text{CO}_2$ ) when tested with phenol red. These factors were given as evidence in support of the theory of the origin of bioelectric potentials as oxidative-reductive systems in flux equilibrium. MARSH (9) showed that the electrical potential difference between apical and basal contacts on the root could be depressed by KCN, and ROSENE and LUND (11) found that the displacement of oxygen with hydrogen around the root also lowered the E.M.F. The readmission of oxygen gave a "rebound" effect in the p.d. of the root which was interpreted as resulting from a greater accumulation of oxidizable material in the apex as compared to the base. BERRY and HOYT (1, 2) observed that an onion root under anaerobic conditions could not be stimulated when direct or alternating current was passed through the root. ROSENE (13) has recently reported the inhibiting effect of KCN on the rate of exudation in excised onion roots. The present paper evaluates the normal respiratory rate of three segments of the root and the problems associated with measurements of this type. There is also included a preliminary estimate of the effects of cyanide and methylene blue on oxygen consumption in these segments.

### Method

The WARBURG manometric method (5) was used for measuring the oxygen consumption of from 34-61 root segments. The dry weights varied from 3-14 mg. and the final values are expressed as cubic millimeters per root per hour. Respiration was also calculated as cu. mm. of oxygen consumed per mg. dry weight per hour ( $Q_{O_2}$ ) but the results were no more reproducible than those reported below. For this reason the former values alone are reported in this paper. Average values obtained in this way were checked on single roots using the SCHOLANDER microrespirometer (15).

The roots were grown as usual in an aerated tap-water aquarium from bulbs 10-15 mm. in diameter. The bulbs were supported on a paraffined wooden board so that the roots could grow freely into the water through holes in the board. In preliminary experiments they were permitted to grow down into open glass tubes with the bulb supported on a flanged end. More

uniform aeration of all roots was obtained when no glass tubes were used. A thermoregulator maintained the aquarium temperature at  $25^{\circ} \pm 1^{\circ}$  C. Roots 30–60 mm. in length were used and a total growth period of  $72 \pm 8$  hours was employed.

Root segments were cut the desired length with small dissecting scissors having a rotating compass point soldered to one blade. For minimum injury the segments were placed immediately on moistened filter paper and transferred to the flasks by spatula. Roots from 8–15 bulbs were used, each flask having the same number of tips from each onion, with either equal lengths or 5, 10, and 15-mm. lengths used in the different flasks for one experimental run. The time required to prepare tissue limited the number of experimental flasks to three. With the temperature of the water bath at  $25^{\circ}$  C. and shaking speed at 100 oscillations per minute, the respiratory rate was constant for three hours or more and then began to decline. Only the rates of oxygen consumption measured during the initial three hours are, therefore, reported in this paper. After the experimental run, roots were dried in an electric oven at  $60\text{--}70^{\circ}$  C. and weighed to 0.5 mg.

## Results

### NORMAL RESPIRATION

Table IA summarizes the results of Warburg manometric measurements made with three different lengths of root tips, as shown in column one. The range of values calculated as cubic millimeters of oxygen consumed per root per hour is given in column four and the mean value and probable error for each segment are shown in column five. From these results it is possible to evaluate and compare the rates of oxygen consumption for the arbitrarily selected segments of root designated in column six. The values as they appear in column seven are obtained by finding the difference between the mean values in column five. The apical 5-mm. root tip has an oxygen consumption

TABLE I

#### NORMAL RESPIRATION OF ONION ROOT TIP A. WARBURG MANOMETERS

LENGTH OF ROOT TIP	NUM- BER OF EXPS.	NUMBER OF ROOTS PER EXP.	MM. <sup>3</sup> O <sub>2</sub> /ROOT/HR. (RANGE OF VALUES)	MM. <sup>3</sup> O <sub>2</sub> /ROOT/HR. (MEAN)	SEG- MENT OF ROOT	MM. <sup>3</sup> O <sub>2</sub> /ROOT/HR. (BY DIFFER- ENCE)	% OF APICAL 5 MM.
I	II	III	IV	V	VI	VII	VIII
mm.					mm.		%
0-5	31	46-61	0.783-1.189	$0.997 \pm .075$	0-5	(0.997)	100.0
0-10	41	36-56	1.349-2.116	$1.678 \pm .144$	5-10	0.681	68.3
0-15	39	34-60	1.870-2.793	$2.255 \pm .137$	10-15	0.577	57.8

#### B. SCHOLANDER MICRORESPIROMETER

0-5	2	1	0.768-1.081	$0.925 \pm .105$	0-5	(0.925)	100.0
0-10	5	1	1.371-1.678	$1.522 \pm .074$	5-10	0.597	64.5
0-15	7	1	1.567-2.351	$1.977 \pm .162$	10-15	0.455	49.2



over 30 per cent. greater than the adjoining 5-mm. length and 40 per cent. greater than the segment 10-15 mm. above the tip (column right). The values thus obtained seem more reliable than actual measurements on root segments with two cut surfaces. It is reasonable to assume that the shortest segments of roots cut 5 mm. above the tip suffered proportionally greater stimulation and protoplasmic injury than those cut 10 to 15 mm. above the tip. If the increase in respiration from stimulation exceeds the decrease due to injury to the cells, the observed differences in respiratory rate of the polar segments would slightly exceed the true values. There is, however, nothing in these data that indicates the nature of the error introduced by cutting.

Results obtained with the Scholander microrespirometer are given under the same headings in Table IB. In order to test the significance of the difference between means shown in column seven, the "t" test was employed (7). This test is particularly useful for small samples and measures the probability level at which the difference in means might occur by chance. If the probability is 5 per cent., the difference is statistically significant and 1 per cent. is highly significant statistically. Both values found here gave a probability of less than 1 per cent. Oxygen consumption of individual roots was 80-86 per cent. of that obtained by the Warburg technique with roots grown on the same days; however, it is clear that the same type of polar distribution of oxygen consumption is obtained. The percentage values listed in the last column agree within 9 per cent. with those derived from Warburg measurements.

It was originally hoped that the use of a large number of segments in each manometer flask would cancel the variations in the respiratory rate of individual roots. Lack of reproducibility was observed, however, and certain environmental factors which might influence these variations were controlled. The aquarium in which the roots were grown was kept at constant temperature and the roots were permitted to develop unrestricted by tubes in an aerated aquarium in order to maintain a reasonably uniform oxygen tension. Some roots were grown in test tubes but results from these were also variable. With 10 per cent. Knop's solution substituted for tap water in the aquarium, the reproducibility of respiratory rates was not improved. It is significant, however, that in experiments set up in duplicate or triplicate on a given day with the same number of roots from a given bulb in each flask, the respiratory rates fell within the approximate limits of error of the method, less than 5 per cent., in 16 out of 20 cases. Of the four exceptions, all of which fell within 10 per cent., two were found in five tests with 5-mm. lengths of root; one in nine tests with 10-mm. lengths and one in six tests with 15-mm. lengths. These results would suggest, therefore, that the sampling for a given determination was adequate to balance out the variations in respiration found in roots of the same bulb and in roots of different bulbs subjected to approximately identical growth conditions. There are no data on the range that might be found in the oxygen consumption of roots from the same bulb but preliminary measurements show that roots reaching a

length of 30 mm. after two days' growth had 35-45 per cent. greater oxygen consumption than roots permitted to grow for four days. Since all roots at least 30 mm. long were used in each experiment and since some of these roots may have been younger than others, it may be that a comparable range of variation might be expected. ROSENE (12) has found that the velocity of water absorption measured as cubic millimeters of water per square millimeter of root surface per hour may show considerable difference in roots from the same bulb.

Growth conditions not controlled from day to day were room temperature and illumination. The temperature varied from approximately 14° to 21° C. and even though the bulbs were immediately above a thermostatically controlled water bath, it may be that they were influenced to some degree by this range in temperature. The roots, on the other hand, were always subject to some change in temperature during the period required to prepare them for study. There is not sufficient data available, however, to permit an accurate analysis of this effect. Since WEINTRAUB (16) has recently shown that light of low intensities inhibits the growth of roots, there is reason to believe that constant illumination of the growth aquarium might lead to more uniform respiration.

#### EFFECT OF POTASSIUM CYANIDE ON RESPIRATION

After a period of normal respiration potassium cyanide, 1/1000 M final concentration, was added to manometer flasks. All of these measurements were made in Warburg manometers, using 40 or more roots in each determination. Results are summarized in table II, where columns three and four give, respectively, the range and the mean with probable error of cubic mm. of oxygen consumed per root per hour. The average oxygen consumption of the same three 5-mm. segments was calculated by difference of the means with the results shown in column six. When the differences of the means were subjected to the "t" test they were found to be highly significant with a probability of less than 1 in 100 that the differences shown would occur by chance. When these rates are compared with the normal rate of oxygen consumption for the corresponding segment, it is seen that the first 5 mm.

TABLE II  
RESPIRATION OF CYANIDE POISONED ONION ROOT TIPS

LENGTH OF ROOT TIP	NUMBER OF EXPS.	MM. <sup>3</sup> O <sub>2</sub> /ROOT/HR. (RANGE)	MM. <sup>3</sup> O <sub>2</sub> /ROOT/HR. (MEAN)	SEGMENT OF ROOT TIP	MM. <sup>3</sup> O <sub>2</sub> /ROOT/HR. (BY DIFFER- ENCE)	% OF NORMAL*
I	II	III	IV	V	VI	VII
mm.				mm.		%
0-5	4	0.341-0.492	0.445 ± .042	0-5	(0.445)	44.6
0-10	4	0.724-0.934	0.819 ± .051	5-10	0.374	55.0
0-15	3	1.234-1.416	1.318 ± .051	10-15	0.499	86.5

\* This figure is the quotient of column VI with the corresponding value in Table IA column VII.

of the root tip has only about 45 per cent. of normal respiration in cyanide, whereas, the 5- to 10-mm. segment and the 10- to 15-mm. segment have 55 per cent. and 86 per cent., respectively, of normal respiration in cyanide. The metabolic activity of the cells in a polar tissue like the root seems, therefore, differentially inhibited by the action of cyanide. These results may be compared to those reported by ROSENE (13) in which Webber had found 41.55 per cent. inhibition of oxygen consumption in onion roots with 0.0025 per cent. KCN. These measurements were made with the Winkler technique over a 48-hour period at 24° C. The length of root tips employed was not specified but the value corresponds closely with our 10-mm. lengths. Webber also obtained reversible inhibition (amount not given) with 0.005 M KCN in both apical and basal segments, using the Warburg method. As COMMONER (4) has emphasized, however, the average percentage inhibition by KCN does not necessarily indicate the relative activity of cyanide-sensitive and cyanide-stable respiration. It is necessary to utilize a range of cyanide concentrations in order to find the maximum inhibition. Under these conditions, comparisons of greater validity can be made.

#### RESPIRATION IN CYANIDE AND METHYLENE BLUE

Since the greatest effect of cyanide was produced on the first 5-mm. segment and the minimum effect on the last 5-mm. segment, it was decided to test the combined action of cyanide and methylene blue on 5-mm. and 15-mm. lengths of root tip. The mean results, with probable errors, from seven experiments in which various sequences were followed are shown in table III.

TABLE III

ONION ROOT TIP RESPIRATION IN CYANIDE AND METHYLENE BLUE

LENGTH OF ROOT TIP	NUM- BER OF EXPS.	RESPIRATION IN CN MB	RESPIRATION IN CN MB	RESPIRATION IN CN MB	RESPIRATION IN MB
		CN	NORMAL	MB	NORMAL
I	II	III	IV	V	VI
mm.		%	%	%	%
0-5	4	125.6 ± 1.68	75.5 ± 5.00	66.7 ± 1.00	109.7 ± 6.01
0-15	3	114.7 ± 3.48	77.4 ± 4.80	73.4 ± 2.60	102.1 ± 5.24

In some cases the cyanide was added previous to the addition of 0.002 per cent. methylene blue or the methylene blue was added before the cyanide. In this way it was possible to show that methylene blue increased the oxygen consumption of the first five mm. of the root tip poisoned by cyanide more than it did in basal segments as shown in column three of table III. The presence of methylene blue in cyanide-poisoned roots, for example, produced an increase of approximately 30 per cent. in respiration of the apical 5 mm., as shown in column four (when compared to column seven of table II). The less sensitive basal region showed essentially no response to the methylene blue since most of the changes seen in the 0-15-mm. segments were probably due to the apical cells. Therefore, under the conditions of these experiments

the cyanide-sensitive respiration of the apical region was approximately 50 per cent. restored to normal by the addition of the methylene blue (from 45 per cent. to 75 per cent.). Methylene blue alone produced a slight elevation above normal respiration, but this increase approaches the limit of accuracy of the Warburg technique. This and the small number of experiments render the increase of doubtful significance.

### Discussion

The oxygen consumption of individual roots might be expected to show considerable variation, not only because of inherent differences, but also because of inconstant growth conditions. The fact that measurements with samples of 40 or more roots failed to compensate for these variations from day to day but were effective on a given day emphasizes the importance of the environmental factors. Since light intensity seems to be at least one factor not rigorously controlled in these experiments, it is interesting to note the rather inconsistent effects of light on respiration of colorless plant tissues reported in the literature. CANNON (3) compared the volume of oxygen removed from water by roots when the shoots were in direct sunlight and in dense shade. Using *Salix* and *Helianthus*, he found that in 69 per cent. of the cases less oxygen was absorbed in light. FÖCKLER (6), on the other hand, found that light accelerates respiration in a variety of colorless plant tissues, especially at high intensities. In no case was a retarding effect observed. The oxygen consumption of *Pistia* roots was reported by RANJAN (10) to be unaffected by light. It is thus apparent that additional work is required before any valid conclusion can be drawn regarding the rôle of light in root respiration in general and especially as it may apply to onion roots.

The effect of temperature on oxidative metabolism is well known. The roots were grown at the same temperature that was used for measuring oxygen consumption but, since the bulbs may have been subject to some cooling and since the preparation of roots for experimentation necessitated a brief exposure to room temperature, an alteration in the mean level of respiration may have occurred from day to day. The data, however, do not permit a valid conclusion on this point. The lack of uniformity encountered in these experiments is not unique since other workers using adequate samples have also observed a wide range of respiratory values. ROSS (14), for example, reported a range of 20 to 30 for  $Q_{O_2}$ 's of *Nitella*, based on one gram wet weight (corresponding to 25 mg. dry weight). This is comparable to the results observed with the onion root.

The differences in oxygen consumption which were calculated for the three 5-mm. segments of root tip and were found statistically to be highly significant might be expected from cytological evidence alone. The apical segment contains the active mitotic cells with few vacuoles and a maximum content of protoplasm while the two more basal segments have the large sap vacuoles, very little meristematic tissue and highly differentiated cells. The

mass of protoplasm becomes progressively less in going from the tip to more basal segments. Thus a greater oxygen consumption does not necessarily reveal a greater intensity of oxidative metabolism in apical cells but rather a greater quantity of metabolizing material. The differential inhibition of oxygen consumption by cyanide suggests, however, that there is a polar difference in the oxidative metabolism of the onion root. This is further borne out by the greater increase in oxygen consumption of the cyanide poisoned root when methylene blue is added to apical segments as compared to longer lengths of root. There is, therefore, direct experimental evidence for the polar difference in oxidative metabolism which has been offered as explanation for the electrical behavior of onion roots under various environmental conditions (1, 2, 8, 9, 11). The ultimate proof of such a polar difference must await, however, more extensive and carefully controlled investigations.

### Summary

1. The average oxygen consumption in cubic millimeters per root per hour, as measured with 34-61 roots by the Warburg manometric method, is 0.997 for 5-mm., 1.678 for 10-mm., and 2.255 for 15-mm. lengths of onion root. Similar results were obtained on individual lengths of root by the Scholander microrespirometer but the average values were 80 to 85 per cent. of the above.

2. There was as much as 50 per cent. variation in day to day values in all lengths of roots studied, but the results agreed within 10 per cent. in experiments set up in duplicate or triplicate on a given day. The maintenance of certain constant growth conditions failed to eliminate the variability but room temperature and illumination were not controlled.

3. A polar difference in cyanide inhibition and in the antagonistic effect of methylene blue for cyanide poisoning was found. This was interpreted as suggesting a fundamental difference in the oxidative metabolism of the different segments of the root.

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# COMPARISON OF RESPIRATION, FREE FATTY ACID FORMATION, AND CHANGES IN THE SPECTRUM OF THE SEED OIL DURING THE STORAGE OF COTTONSEED

LILLIAN KYAME AND A. M. ALTSCHUL

(WITH FIVE FIGURES)

## Introduction

The phenomenon referred to as seed deterioration is a complex of a number of different biological and chemical processes. Although a number of these processes have been independently investigated, there is little available information on the relationships existing between them. For example, while it is known that increased moisture content results both in increased lipolytic and respiratory activity, it has not been established whether these two activities are independent manifestations of increased moisture content or whether they are functionally related. For this reason it was of interest to follow lipolytic activity and changes in the seed pigments in those seed samples used for the respiration measurements reported in the preceding publication of this series (7).

## Materials and methods

The 3-pound lots of seeds that had been set aside for the respiration measurements (7) were used as a source of material for free fatty acid determinations. One portion of the seed oils extracted from samples periodically withdrawn from containers was analyzed for free fatty acid content, while spectrophotometric measurements were made on another portion. The free fatty acid analyses were carried out according to the method previously described by KARON and ALTSCHUL (6), and the spectrophotometric measurements as described by ALTSCHUL and coworkers (1).

In all that follows, moisture content is expressed on the basis of the total wet weight of the seed, and the amount of free fatty acids in terms of percentage of oleic acid. Spectrophotometric measurements were conducted in a Coleman double monochromator spectrophotometer with an absorption cell thickness of 13 mm.; absorption data refer to a solution containing 1 ml. of oil in a total volume of 50 ml., carbon tetrachloride being used as the solvent.

## Free fatty acid formation

In a previous publication (6), it was shown that the rate of lipolysis in cottonseed can be evaluated by the use of the autocatalytic-type equation:

$$dF/dt = k(F)(100 - F) \quad (1)$$

where

$F$  = the percentage of free fatty acids formed,  
 $100 - F$  = the percentage of residual unhydrolyzed fat,  
 $t$  = the number of days of storage.

Thus the numerical value of the rate constant,  $k$ , in equation (1) quantitatively characterizes the course of free fatty acid formation in cottonseed.

From the data obtained by periodic free fatty acid analyses upon 18 lots of seed, values of the rate constant,  $k$ , were determined (table I).<sup>2</sup> It will be noted that there is no evaluation of  $k$  for three of the seed lots. The rate of formation of free fatty acids in lot 205a was too low to be accurately measured. The seeds in lots 204a and 204b gave hydrolysis patterns which

TABLE I

THE EFFECT OF MOISTURE ON THE RATE CONSTANT,  $k$ , FOR THE FORMATION OF FREE FATTY ACIDS IN STORED COTTONSEED

SAMPLE	VARIETY	DATE OF HARVEST, 1942	MOISTURE CONTENT	MOISTURE TREATMENT	$k$
			%		<i>reciprocal of days</i>
104b	Delfos 3506	Aug. 29	10.0	Lowered	$1.7 \times 10^{-5}$
105b	"	Oct. 3	10.6	None	2.2 "
104a	"	Aug. 29	12.0	None	2.0 "
105a	"	Oct. 3	13.1	Raised	5.0 "
105f	"	"	14.2	Raised	8.7 "
105f'	"	"	14.9	Raised	13.6 "
105e	"	"	16.8	Raised	17.6 "
205a	Coker's 200, strain 1	Sept. 22	12.5	Raised	.....
204b	"	"	12.5	Lowered	.....
204a	"	"	13.7	None	.....
206a	"	Oct. 22	14.7	Raised	6.0 "
205e	"	Sept. 22	15.9	Raised	13.6 "
305b	Oklahoma Triumph, 0-52-12	Oct. 1	10.6	None	1.4 "
306b	"	Oct. 28	10.6	None	2.4 "
307a	"	Nov. 28	12.9	Raised	3.2 "
306a	"	Oct. 28	13.1	Raised	2.6 "
304a	"	Sept. 22	13.3	Raised	3.6 "
308a	"	Dec. 28	15.7	None	12.9 "

were fundamentally different from those of the other seed samples investigated.

The values of  $k$  are plotted as a function of moisture content in figure 1. The experimental points for the samples in the 100 series all fall on one curve, whereas the points for the samples in the 200 and 300 series fall on a second curve which is lower than the first. In this respect the results of the lipolysis rate determinations resemble very closely those of the respiration measurements on the same seed sample (7), since in both instances a higher level of activity at comparable moisture contents was exhibited by the seeds in the 100 series.

The curves in figure 1 lend themselves to further analysis by the use of the following autocatalytic-type equation:

$$dk/d(H_2O) = k'(A - k) \quad (2)$$

where  $k$  = lipolysis rate constant,  
 $(H_2O)$  = percentage moisture in the seed,  
 $k'$  = constant relating moisture content to lipolysis  
 rate constant  
 $A$  = arbitrary constant.

Upon integration, equation (2) becomes

$$\log k/A - k = A k' (H_2O)/2.3 + \text{constant.} \quad (3)$$

If the equation is applicable to the curves in figure 1, there should exist a value of  $A$  for which a plot of  $\log k/A - k$  against  $(H_2O)$  will give a straight

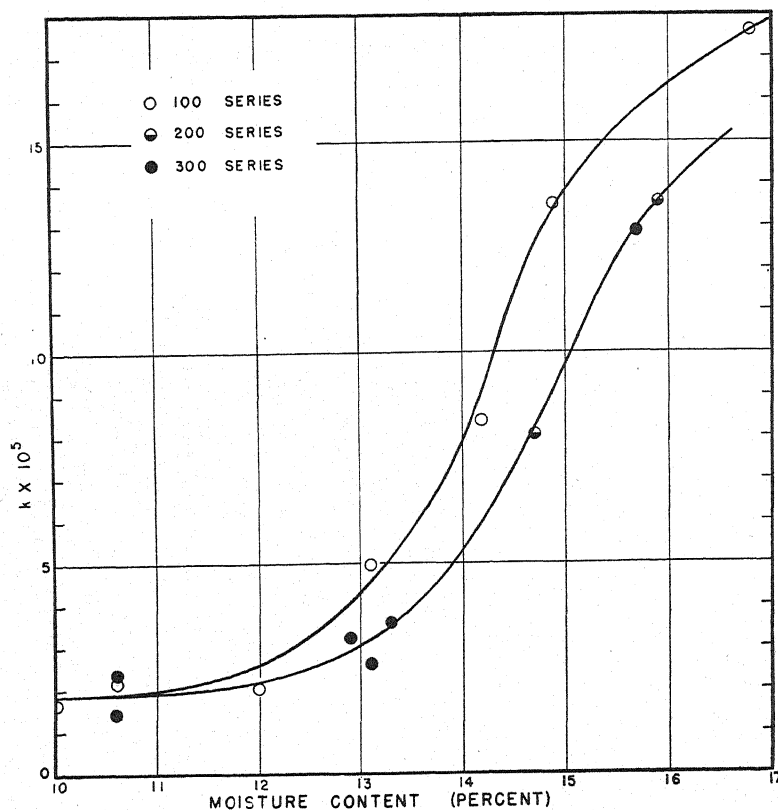


FIG. 1. The effect of moisture content on the lipolysis rate constant of cottonseed.

line with a slope equal to  $A k'/2.3$ . When a value of  $50 \times 10^{-5}$  was chosen for  $A$ , it was found that straight lines could be obtained to represent in a linear manner the effect of moisture on a function of the lipolysis rate constant. As is shown in figure 2, the data for the seeds in the 100 series fall on one straight line in the range of 11–16 per cent. moisture and the data for the seeds in the other two series fall on a second straight line. In order to compare the properties of the present seed samples with those used in a previous investigation (6), the rate constants for these seed samples were

treated in the same manner and here again a straight line was obtained<sup>1</sup> (curve 1, figure 2). The slopes of the lines in figure 2 are 0.27 per one per cent. moisture for the seeds in the 200-300 series, 0.33 for those in the 100 series, and 0.43 for the "Delfos" (6) seeds harvested in 1941.

It is interesting to note that the methods of analysis used to express respiration intensity and rate of hydrolysis as linear functions of the mois-

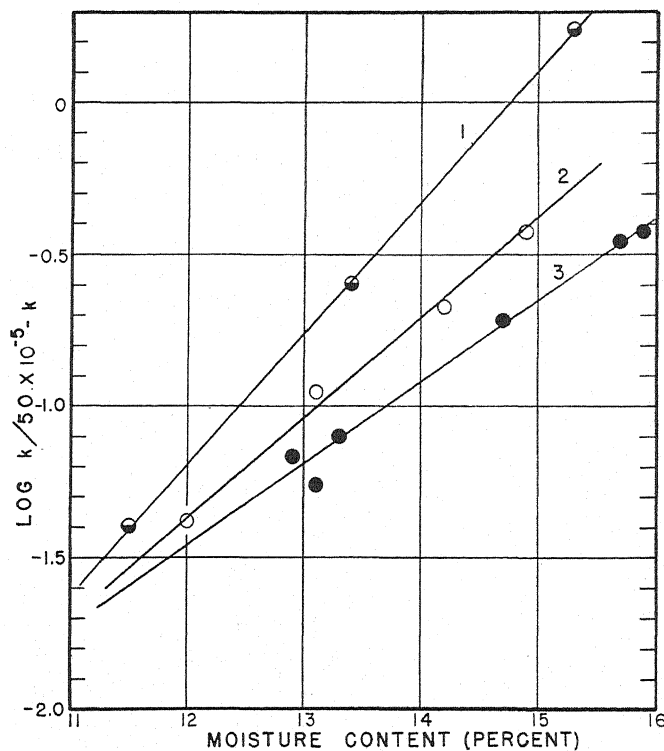


FIG. 2. The effect of moisture content of cottonseed on a function of the lipolysis rate constant.

1. "Delfos" variety of cottonseed (6), harvested in 1941.
2. 100 series of seeds, "Delfos" variety harvested in 1942.
3. 200 and 300 series of seeds, "Coker's" and "Oklahoma Triumph" varieties, harvested in 1942.

ture content indicate different types of relationship between seeds in the 100 series and the 200 and 300 series. From the respiration data it was found that the seeds in the 200 and 300 series exhibited the same sensitivity to increase in moisture as did the seeds in the 100 series, manifested by the fact that the slopes of the two lines representing these series were the same (7). The two series differed, however, in that the 100 series respired at a

<sup>1</sup> In order to compare previous results (6) with those reported in this investigation, it was necessary to convert the values for the percentage moisture content from the dry basis used previously to a wet basis as reported in this publication.

higher level of intensity. The hydrolysis data, on the other hand, revealed that the seeds of the 100 series exhibited a higher level of lipolysis and also displayed a higher sensitivity to increased moisture than those in the 200 and 300 series. In both lots practically the same rate of lipolysis occurred at a moisture content of 11 per cent., but great differences were observed at higher moisture values.

It is not immediately apparent whether this difference between the rate of respiration and lipolysis represents an intrinsic biological phenomenon or whether the relationship that seems to exist is a result of the methods

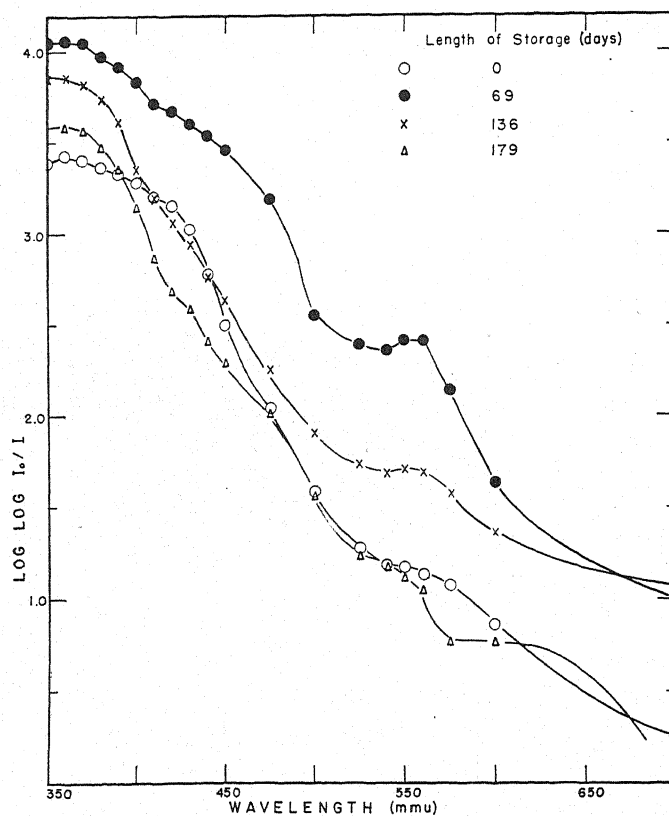


FIG. 3. The variation in the absorption spectrum of extracted oils from sample 205e during storage.

of mathematical analysis used. For all practical purposes the respiratory intensity, defined as the average respiration rate of the seed, is independent of the character of the respiratory pattern. On the other hand, the hydrolysis-rate constant,  $k$ , is determined by the rate of increase in free fatty acids in the seeds as a function of the length of storage. It is quite possible, therefore, that two such different constants would not respond in the same manner to mathematical analysis. Unfortunately, because of the irregular nature of the respiratory patterns, it is not possible to use any but an aver-

aging process to arrive at a quantitative value for respiratory intensity. On this basis there cannot be obtained a mathematical measure of the sensitivity of respiratory intensity to moisture which will be wholly comparable to that for lipolysis.

#### Absorption spectra of solvent-extracted oils

When cottonseed flakes or meats are extracted with fat solvents, there are extracted along with the oil a considerable quantity of pigments whose nature has been the subject of many investigations. As a result of such investigations, a new class of pigments peculiar to cottonseed has been

TABLE II

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560  $m\mu$  OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 100 SERIES

SAMPLE	LENGTH OF STORAGE	Log $I_0/I$	SAMPLE	LENGTH OF STORAGE	Log $I_0/I$
	<i>days</i>			<i>days</i>	
104b	0	0.012	104a	0	0.021
	87	0.010		52	0.012
	232	0.019		115	0.007
	367	0.032		218	0.014
105a	0	0.005	105b	367	0.037
	27	0.009		0	0.011
	122	0.011		71	0.010
	328	0.021		160	0.008
105f	0	0.013	105f'	342	0.026
	66	0.015		29	0.018
	120	0.016		120	0.017
	337	0.016		156	0.011
105e	27	0.036		337	0.028
	62	0.032			
	111	0.012			
	147	0.020			
	223	0.093			

disclosed. Of these, gossypol, a yellow pigment, is the most abundant and the best known. BOATNER *et al.* (3, 4) have shown, however, that at least four pigments besides gossypol exist in cottonseed and that some of these, at least, are interrelated.

Much of the pigmented material in the cottonseed is localized in structures known as pigment glands distributed throughout the embryo (8). All of the pigments so far isolated have been found in these glands, from which they can be readily extracted by use of suitable solvents (5). There are solvents, however, that are incapable of breaking up the glandular structure. These solvents are able to extract only those pigments that occur outside the glands or are contained in the small number of ruptured glands present in cottonseed flakes or ground cottonseed. An example of solvents of the latter type is Skellysolve F (low-boiling petroleum ether).

In the experiments reported above, petroleum ether was used to extract



TABLE III

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560  $m\mu$  OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 200 SERIES

SAMPLE	LENGTH OF STORAGE	Log $I_o/I$	SAMPLE	LENGTH OF STORAGE	Log $I_o/I$
	<i>days</i>			<i>days</i>	
204a	0	0.030	204b	0	0.041
	69	0.036		31	0.031
	142	0.051		67	0.033
	212	0.071		140	0.048
				210	0.074
205a	0	0.044	206a	0	0.017
	29	0.025		56	0.013
	179	0.025		109	0.013
	338	0.064		244	0.071
				316	0.043
205e	0	0.014			
	69	0.258			
	186	0.048			
	179	0.011			

the seed oil which was then analyzed for free fatty acids. On aliquots of the same extracted oil, periodic spectroscopic determinations were also made. As an example of the type of results obtained in the spectroscopic analyses, the data for sample 205e are given in figure 3. It will be noted that as storage progresses there is a change in the spectra of the oils. At 560  $m\mu$ , the point of maximum light absorption of the pigment gossypurpurin (3), there is first an increase in absorption, which is followed by a decrease as the storage is continued. The light-absorption value after 179 days of storage is very close to the original value. A similar pattern of

TABLE IV

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560  $m\mu$  OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 300 SERIES

SAMPLE	LENGTH OF STORAGE	Log $I_o/I$	SAMPLE	LENGTH OF STORAGE	Log $I_o/I$
	<i>days</i>			<i>days</i>	
304a	0	0.007	305b	0	0.020
	32	0.022		71	0.019
	91	0.026		267	0.070
	146	0.038		339	0.053
	225	0.100			
	327	0.105			
306a	0	0.032	306b	0	0.018
	56	0.030		68	0.043
	110	0.040		323	0.068
	212	0.084			
	317	0.058			
307a	0	0.022	308a	0	0.021
	64	0.023		55	0.072
	144	0.043		133	0.088
	282	0.079		237	0.012

change is observed in the absorption at 360  $m\mu$ , a point of maximum light absorption of gossypol (3). Again there is an increase in the light absorption during the initial stages of storage followed by a decrease as storage is continued.

The effect of storage on the light absorption of the oil at 560  $m\mu$  is given in tables II, III, and IV. Inasmuch as it has already been shown that the 200 and 300 series of seeds behave similarly with respect to both their respiration behavior and lipolysis rates, it was of interest to examine the experimental data to determine whether there might be a similar correlation between the

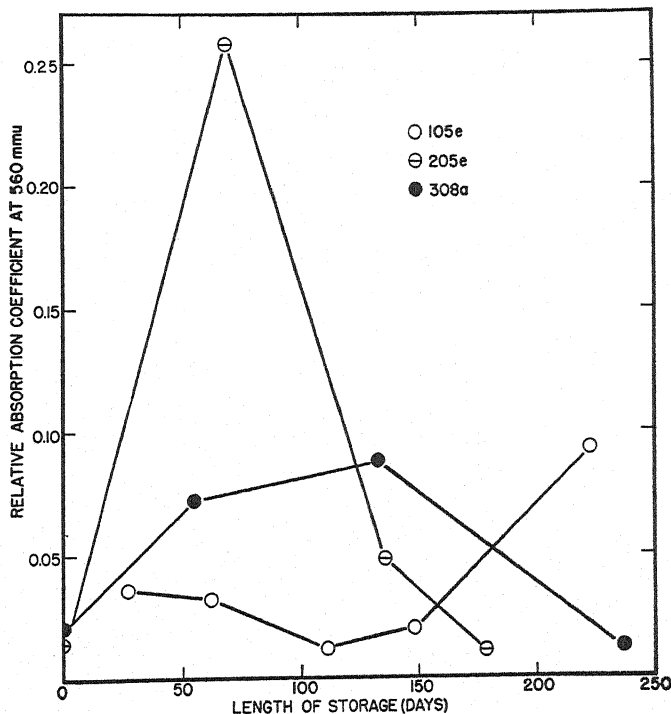


FIG. 4. The effect of storage on the relative light absorption coefficient at 550  $m\mu$  in the case of high-moisture seeds.

changes in the light absorption of the oils and other seed characteristics. Accordingly, the relative absorption coefficient at 550  $m\mu$  for the sample having the highest moisture content in each series was plotted against the days of storage (fig. 4). It is significant to note that the patterns of change in the 200 and 300 series are similar in that they both exhibit a maximum during the storage period. On the other hand, the pattern in the 100 series is reversed; i.e., there is a minimum in absorption during the same storage interval.

As yet too little is known concerning the chemistry and biochemistry of cottonseed pigments to permit one to draw any significant conclusions from the above-mentioned data. Inasmuch as Skellysolve F does not rupture the pigment glands, the observed changes in light absorption may be due

both to changes in the relative pigment concentrations in the seeds and glands and to changes in the amount of Skellysolve F-soluble pigmented material which occurs outside of the glands. As soon as more information concerning cottonseed pigments is available, it should be possible to interpret data of this type with more certainty and to correlate them with other seed characteristics.

#### Immature seed

In the preceding publication (7) it was indicated that the samples of immature seed, 204a and 204b, exhibited a much higher level of respiration than did any of the other samples. An examination of the course of lipolytic activity in these same seeds indicated that they differed materially also

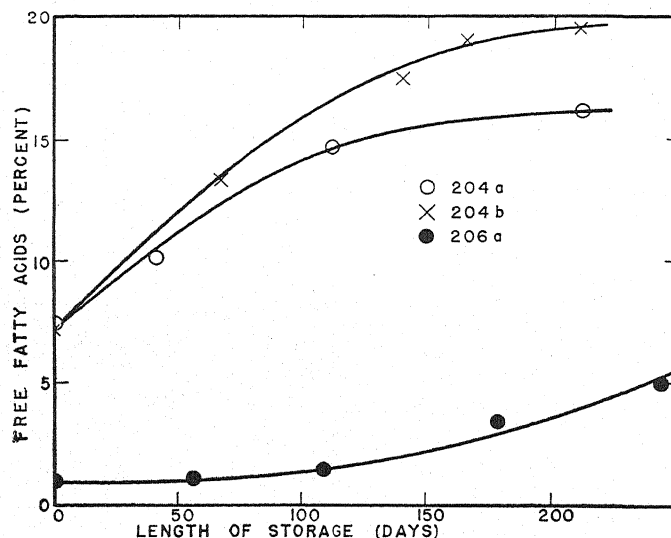


FIG. 5. Free fatty acid formation during the storage of samples 204a, 204b, and 206a.

from the other samples with respect to their rate and pattern of formation of free fatty acids. Whereas all of the other seed samples exhibited a normal accelerating rate of lipolysis as storage continued (6), the immature seeds exhibited the highest rate of lipolysis at the beginning of the storage period. As is shown in figure 5, where the curves for the rate of development of free fatty acids in samples 204a and 204b are compared to a curve for normal seeds of about the same moisture content, the rate of formation of free fatty acids in the immature seeds at the end of 200 days' storage was practically zero. On the other hand, the normal seeds were just beginning to display an appreciable rate of lipolysis at that time.

#### Discussion

There would appear to be two alternative explanations of the parallel relationship which exists between the vigor as reflected by lipolytic rate and the vigor as reflected by respiratory rate of the three series of seeds investigated: (1) the two processes depend on the same biological systems, or (2)

they reflect a general condition in the seeds whereby all types of enzymatic processes are maintained at a similar level of activity. The former explanation, *i.e.*, that lipolysis and respiration are functionally related, is probably not correct, in view of the differential effect of inhibitors on these two processes as shown in another publication (2).

It seems evident that the intensity of respiration in seeds is dependent on the amounts of the respiratory and associated enzyme systems that are present in an active state and on the rate of release or production of additional active enzymes (7). Similarly, the rate of lipolysis is determined by the initial concentration of active lipases and by the ability of the cell systems to release additional lipolytic enzymes as the storage progresses. The fact that seeds which exhibit equal respiratory activities (the 200 and 300 series) also exhibit equal lipolytic activities is a strong indication that seeds may be characterized by their state of "biological vigor." At least for the two variables, lipolysis and respiration, vigor with respect to one is accompanied by equal vigor with respect to the other. On the basis of this reasoning it is not inconceivable that the seeds in the 200 and 300 series would show similar behavior and lower vigor than those in the 100 series with respect to other properties such as germination, strength, and rate of growth of the seedlings. There is, however, no evidence in the present work which substantiates such a generalization.

That both respiration and lipolysis equally reflect the vigor of cotton seeds is a fact of great practical importance in commercial storage. On the basis of this fact it would be reasonable to predict that seeds whose respiration intensity is high—that is, seeds that heat readily when stored in bulk would, even when heating is controlled by air circulation, undergo more intensive lipolysis than seeds of equal moisture content but lower respiratory activity. Thus, the measurement of the heat resulting from respiration should be a useful criterion in predicting the storage behavior of seeds.

The behavior of immature seeds is entirely different from that exhibited by mature seeds. In immature seeds, the enzyme systems have not reached the state of dormancy which characterizes them in mature seeds. For this reason immature seeds are more sensitive to moisture and respire at a higher intensity than do mature seeds. The course of lipolysis in immature seeds indicates that the hydrolytic activity is due exclusively to enzymes already present in the active state and that no production of additional active enzymes takes place during the course of storage. Actually, it would seem that the enzyme systems responsible for free fatty acid formation are rendered less and less active as the storage of immature seeds progresses. In effect, then, the maturation process which is interrupted by early harvesting of the cotton, is continued during storage.

### Summary

1. Samples of cottonseed that were used in the respiration investigations reported in a previous paper (7) were analyzed for free fatty acid content, and the lipolysis-rate constant,  $k$ , was determined for each of the samples.

2. When the lipolysis constants were plotted as a function of moisture content, it was found that the constants for the "Delfos" seed all fell on a smooth curve and that constants for the "Coker's" and "Oklahoma Triumph" varieties fell on a second smooth curve which was lower than the "Delfos" curve.

3. It was found that the lipolysis constant can be converted into a linear function of the moisture content of the seed by the use of the differential equation:

$$dk/d(H_2O) = k' k (A - k)$$

where

$k$  = hydrolysis rate constant,  
 $(H_2O)$  = percentage moisture in the seed,  
 $k'$  = constant relating moisture content to lipolysis rate constant  
 $A$  = arbitrary constant.

When  $A$  is given the value of  $50 \times 10^{-5}$ , the log of  $k/A - k$  becomes a linear function of the moisture content.

4. The slopes of the lines denoting lipolysis as a linear function of moisture content were found to be 0.27 unit per one per cent. moisture for the "Coker's" and "Oklahoma Triumph" seeds and 0.33 unit for the "Delfos" seeds.

5. A variation of the spectrum of solvent-extracted oils during the storage of cottonseed was found to exist. The highest moisture samples of the "Coker's" and "Oklahoma Triumph" varieties showed a common pattern which was in turn different from that exhibited by "Delfos" variety.

6. The pattern of lipolysis for immature seeds was shown to be different from that for normal cottonseed in that the rate of formation of free fatty acids decreased with length of storage.

7. The "Delfos" seeds exhibited more vigor both with respect to lipolysis and respiration than did the seeds in the "Coker's" and "Oklahoma Triumph" varieties.

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## OSMOTIC PRESSURE DETERMINATIONS WITH ISOLATED PROTOPLASMIC PROTEINS

J. LEVITT

(WITH SIX FIGURES)

LOEB (9), KUNTZ *et al.* (6), SCATCHARD *et al.* (11), and others have made use of osmotic pressure measurements to calculate the molecular weights of pure proteins such as gelatin, crystallized enzymes, and crystallized serum albumen. Protoplasmic proteins have not been investigated. Yet it would be of some interest to know the average molecular weight of a plant's protoplasmic proteins. Such information is essential if, for instance, one is to test theories of protein splitting at low temperature. Curves obtained by plotting pressure against concentration also yield information about the relative hydration ("bound water") of the protein, for as the concentration increases, a larger and larger portion of the pressure is due to the hydration rather than the number of molecules present. The adaptation of a plant to unfavorable environmental conditions is frequently ascribed to increased hydration of proteins (4). Evidence for such theories has heretofore been indirect; *e.g.*, from measurements of protoplasmic viscosity or from the properties of plant juices which come mainly from the vacuole rather than the protoplasm (7).

### Methods

#### ISOLATION OF PROTEINS

In the following investigation, potato tubers (Russet variety) were used, partly because of the constant availability of material and partly because no proteins other than protoplasmic are to be found (except some crystals in cells immediately below the periderm which were removed with the peel).

One reason why protoplasmic proteins have not been investigated is the difficulty in obtaining them in the undenatured state. Preliminary experiments were therefore made with the filtrate from Waring blender mash. The proteins were precipitated from the filtrate with  $(\text{NH}_4)_2\text{SO}_4$ , filtered off, then dialyzed. The importance of pH soon became evident. If no precautions were taken to regulate the pH, very little protein was obtained. If enough  $\text{K}_2\text{HPO}_4$  solution was pipetted into the blender (before mashing) to obtain a pH of about 7.0 in the filtrate, a considerable amount of protein was obtained in the soluble state. If, then, enough  $\text{NH}_4\text{OH}$  was added to neutralize the  $(\text{NH}_4)_2\text{SO}_4$  before precipitating the proteins, 80-90 per cent. of the extracted protein remained in the soluble state. Solubility was determined by centrifuging for 5 minutes at 600 g. then weighing the precipitated and unprecipitated proteins.

There are some disadvantages in the use of the Waring blender. Large quantities of material are needed, a relatively small fraction of the cells is broken open, only succulent tissues can be mashed, and denaturation of pro-

teins may possibly occur due to the frothing and agitation. Consequently this method was discarded in favor of freeze-drying the tissues followed by grinding in a miniature Wiley mill and extraction with  $K_2HPO_4$ .

Since FLOSDORF and MUDD's (2) original work, many modifications of the "lyophile apparatus" have been described (1, 5, 8, 10, 12). The commercial "cryoscopic dryers" avoid the need of dry ice (except for preliminary freezing) by using a desiccant that can be regenerated.

All the above models were designed primarily for drying liquids. Even those that use the wider-necked flasks are not easy to fill and empty when pieces of tissue are used. Furthermore, the relatively close packing of the tissues does not favor rapid drying. After using a "lyophile apparatus" for some time, it was discarded for the following simpler, more efficient, less time-consuming method.

The tubers were cut into slices 2-3 mm. thick, wiped dry, then frozen in dry ice. When completely frozen, they were arranged in layers in a vacuum desiccator between alternating layers of  $Al_2O_3$ . The desiccator was evacuated to a pressure well below 1 mm. then transferred to a cold room at 0-5° C. After 24 hours, the potato slices were dry and pith-like in texture. If the slices are permitted to melt before drying is complete (due to insufficient evacuation or  $Al_2O_3$ ), the melted part when dry is horny hard. Consequently, there is no difficulty in determining whether the tissues were really dried in the frozen state. The method is uniformly successful if a good high-vacuum grease is used and if there is at least 10 times as much  $Al_2O_3$  as the water content of the tissues. Ringing the desiccator joint with Apiezon is a useful precaution to insure maintenance of the vacuum. One desiccator can be used to dry as much as 400 grams of potatoes, containing about 300 grams of water. It is, of course, not necessary to bury the slices in the desiccant, but this is a convenient and simple method where large quantities are to be dried.

The dried tissues were ground in a Wiley mill until they passed the finest screen (60 mesh). Ten grams were then extracted with 35 ml. of a solution of 0.2 M  $K_2HPO_4$  containing 0.01 M KCN. The KCN was included to inhibit enzyme action. It at least prevented the production of the dark oxidation product which was otherwise produced and adsorbed by the proteins. Precipitation was obtained by adding 15 gm.  $(NH_4)_2SO_4$  and 2 ml. of 0.75 N  $NH_4OH$  to 30 ml. of extract. The pH of the solutions was always between 7.0 and 7.6. The precipitated proteins were filtered off with hard filter paper, from which they were easily scraped without removing the cellulose fibers from the paper. The quantity of protein obtained was 1.3 to 2.0 per cent. of the dry matter of the tubers. Further extraction with alkali failed to yield any more proteins precipitable with  $(NH_4)_2SO_4$ .

#### MEASUREMENT OF OSMOTIC PRESSURE

Though indirect methods can be used, and are often preferable when working with substances of low molecular weight, in the case of proteins the

direct method is simpler and more accurate. Semipermeable membranes are easily constructed from collodion. Since the protein comprises a small percentage of the dry matter, and since the osmotic pressure of protein solutions is low enough to require high concentrations, a micromethod must be used. Furthermore, the crude protein obtained contains not only the structural proteins but all of the enzymes as well; consequently precautions must be taken to reduce chemical changes to a minimum. This requires maximum speed of dialysis and low temperature. Fortunately, the use of a micromethod results in a high speed of dialysis due to the large specific surface and the small amount of substance that has to dialyze away. In an attempt to speed up the process still further, electrodialysis was tried, but the proteins were at least partially denatured by the weakest current that was capable of reducing the time of dialysis.

The microcups were made as follows: glass rods 3 mm. in diameter were dipped into a collodion solution (10 gm. parlodion dissolved in 50 ml. absolute ethyl alcohol, 50 ml. ether, 1 ml. glacial acetic acid). After draining for about 45 seconds they were allowed to dry for 10 minutes in the inverted position. They were then immersed in 95 per cent. alcohol, an incision was made around the rod 6 cm. from the base, the cups slipped off, and stored in the 95 per cent. alcohol overnight or longer.

The manometers were constructed from 1-mm. bore capillary tubing (fig. 1). Pressure was regulated by means of a screw clamp attached to the rubber tubing at the base of the manometer. A small bulb was blown in the short arm of the manometer just opposite the zero mark on the long, graduated arm. This served the dual purpose of having a relatively large volume of air between the mercury and the microcup, and of keeping the level of the mercury in the short arm practically stationary.

The tip of the manometer was drawn out to a diameter of about 3 mm. While in the 95 per cent. alcohol, the microcups were plastic. Consequently, they could easily be forced onto the tip of the manometer, after which they were immersed in water. This hardened them in the molded shape. The capacity of a cup (up to the fitted neck) was about 0.2 ml. They were now ready to be filled with the protein precipitate, following which each cup was attached to a manometer and a rubber sleeve slipped over the connection. If the cups had been firmly forced onto the manometer, they would now stand at least three atmospheres of pressure (higher pressures have not been tried) for hours without rupturing, coming loose, or even stretching.

The whole apparatus was set up at  $+2^{\circ}\text{C}$ . Enough protein was obtained from 10 gm. of dry matter to set up five or six manometers simultaneously. The precipitate was transferred to the cups by means of a glass rod 1-2 mm. in diameter. Tight packing was achieved by centrifuging for one-half minute. Dialysis of the  $(\text{NH}_4)_2\text{SO}_4$  was quite rapid. Tests of the dialysate with  $\text{BaCl}_2$ , as well as measurements of the electric current conducted by the protein, revealed that all the  $(\text{NH}_4)_2\text{SO}_4$  (as far as can be detected by these methods) had dialysed away within 12 hours even when highly

concentrated protein gels were used. To be certain that equilibrium had been reached, the standard procedure adopted was to allow 24 hours, during which three changes of water were used (tall jars were found to be as satisfactory as running water since the dense  $(\text{NH}_4)_2\text{SO}_4$  solution sank to the bottom leaving almost pure water in contact with the membranes). Of course, it was not necessary to test for the end point since this was revealed

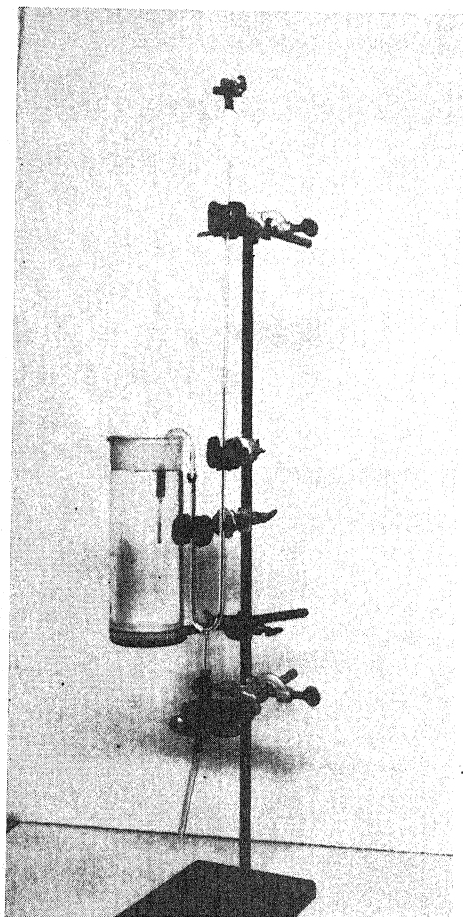


FIG. 1. Manometer with microcup attached for dialysis and osmotic pressure measurement.

by the pressure reading on the manometer. The initial pressures were large, due to the  $(\text{NH}_4)_2\text{SO}_4$ , consequently the manometers were closed by screw clamps for the first few hours to prevent the mercury from going over the top and the protein from taking up too much water. The pressure then dropped, finally reaching a steady rate of drop of 1–5 mm. per hour. A perfectly constant pressure was never attained due to the slow solution and diffusion of the compressed air above the protein. But the change was so

gradual as to produce no significant error. At the end of 24 hours, readings were made, the proteins transferred to weighing bottles (by squeezing them out of the cups like toothpaste from a tube) and dried at 100–110° C. to determine the moisture content.

### Results

In order to test the accuracy of the method used, determinations were made with a pure protein whose characteristics are already well known—crystallized bovine plasma albumin (11). Figure 2 shows the curve obtained

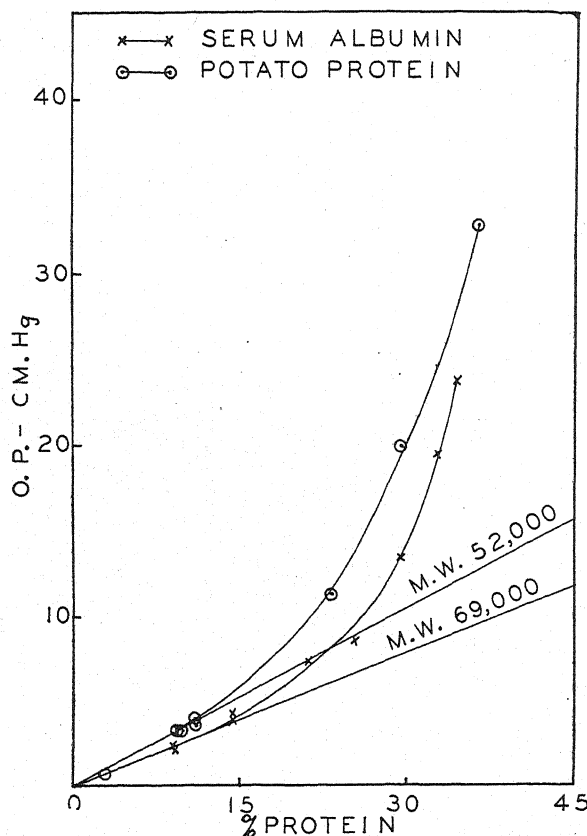


FIG. 2. Osmotic pressure curves for crystallized bovine serum albumin and protein from potato tubers.

with this protein. From the formula  $M = \frac{gRT}{PV}$ , the molecular weight can be calculated, using the lowest values, which are on the extrapolated, straight line portion of the curve. The value for  $V$  (the volume of solution containing  $g$  grams protein) was calculated by adding the weight of the water to half the weight of the dissolved protein. That this yields a sufficiently reliable result was indicated by dissolving 1 gram albumin in 9 ml. water. The volume of the solution was 9.6 ml.

From the curve in figure 2, the molecular weight of the albumin was calculated to be 69,000—the same value as obtained by SCATCHARD *et al.* (11). The average molecular weight of the proteins obtained from a potato tuber was similarly calculated to be 52,000 (fig. 2). The tuber had been kept at 0° C. for some time, and was tested in February. Since the potato proteins consist of a mixture of a large number of different proteins, this average molecular weight cannot be constant, such as the value for the single, pure bovine plasma protein.

The purpose of the next experiment was to determine whether or not the osmotic pressure curve is the same for proteins from growing tissues as for

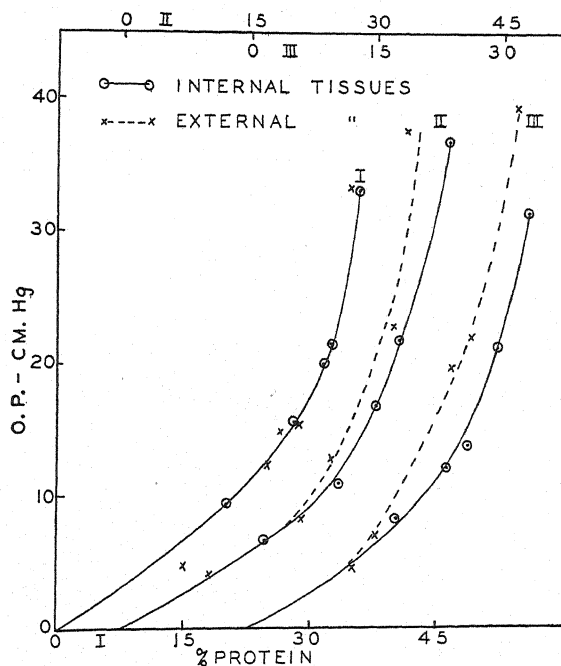


FIG. 3. Osmotic pressure curves for proteins from internal and external tissues of potato. Tubers were peeled then allowed to form a new periderm at 25° C. for 32 hours (I), 2 days (II), and 7 days (III), respectively.

proteins from non-growing tissues. To obtain tissues in the growing state, tubers from the cold chamber were peeled and placed in a large Petri plate over a layer of water at about 25° C. After 3–6 days, microscopic observation revealed the presence of a phellogen layer several cell layers below the surface. The tubers were again peeled, both the new peel (which we will call external tissues) and the remainder of the tuber (internal tissues) were freeze-dried, ground and extracted as described above. Osmotic pressure curves for both sets of tissues are shown in figure 3.

With the two longer exposures to room temperature, the curves diverge markedly at the higher concentrations and converge at the lower. Obviously, there is no appreciable difference in the average molecular weight of



the proteins from the growing and dormant tissues. The divergence at the higher concentrations indicates a higher hydration of the proteins from the growing tissues.

Figure 4 shows the results for control (unpeeled) tubers which were also kept at 25° C. for 6 days. The relative humidity, however, was that of the room in order to prevent any sprouting. At the end of the period, the tubers were peeled and the peel was discarded; the "external tissues" were then removed and compared with the "internal tissues" as in the case of the tubers that were permitted to form phellogen. In two of the three experiments, there is again no difference in average molecular weight of the proteins from the two regions. In this case, however, the curve for the proteins from the

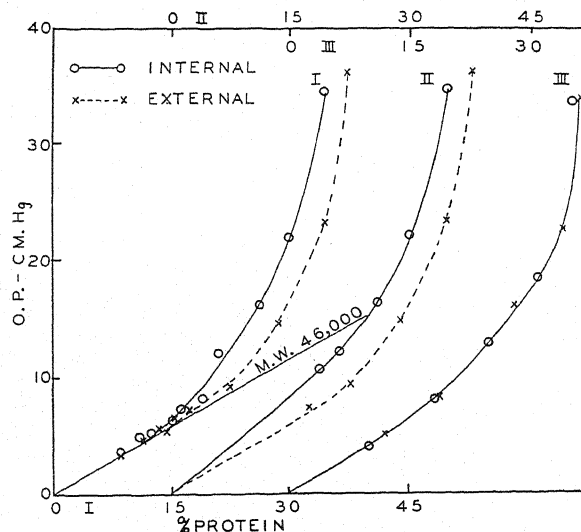


FIG. 4. Osmotic pressure curves for proteins from internal and external tissues of potato. Unpeeled tubers were kept at 25° C. for 6-7 days.

internal tissues rose more steeply than that from the external tissues in two of the three experiments. In the third experiment there was no difference.

Since the controls were not kept at the same relative humidity as the tubers that formed phellogen, in the next experiment the effect of this factor was investigated. Tubers were half peeled and then kept in the nearly saturated atmosphere at 25° C. Only the external tissues from the two regions were compared. Figure 5 shows that there is no appreciable difference between the osmotic pressure curves for the proteins from the peridermed and non-peridermed external tissues. It was noticed, however, that the unpeeled part of the tubers had begun to sprout. Consequently, neither region could be considered dormant.

These results pointed to the desirability of following the changes in the same tissues. Consequently, in the next tests, both external and internal tissues were investigated before and after phellogen formation. Tubers from the cold chamber were cut in half across the short axis. One apical

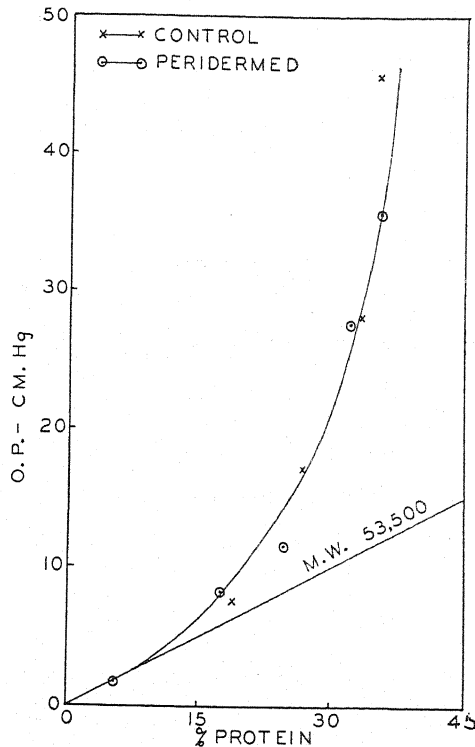


FIG. 5. Osmotic pressure curves for proteins from external tissues of half peeled tubers kept at 25° C. for 6 days. The peeled half formed periderm during this time,

and one basal half from different tubers were then separated into external and internal tissues. The other halves were peeled and allowed to form phellogen. Figure 6 shows the results obtained. They agree with the results reported above, in showing a steeper rise for proteins from external tissues in the peridermed tuber halves. The curves for the proteins from the peridermed external tissue are also steeper than those for the proteins from the control external tissues. This difference is solely due to a difference in average molecular weight. Thus the ratios of the pressures in the first set of curves at 9 and at 33.7 per cent. (the two extremes for the phellogened exter-

TABLE I

CHANGE IN QUANTITY OF PROTEINS ON TRANSFER OF TUBERS FROM 0° C. TO 25° C. FOR 6-7 DAYS (MG. PROTEIN PER GM. DRY MATTER)

TREATMENT	INTERNAL TISSUES		EXTERNAL TISSUES	
	0° C.	25° C.	0° C.	25° C.
	mgm.	mgm.	mgm.	mgm.
Peeled and allowed to form new periderm	14.1	19.8 <sup>a</sup>	17.5 <sup>b</sup>	19.2
	13.0	16.8	14.6	18.1
Unpeeled	15.2	16.0	16.6	21.8 <sup>c</sup>

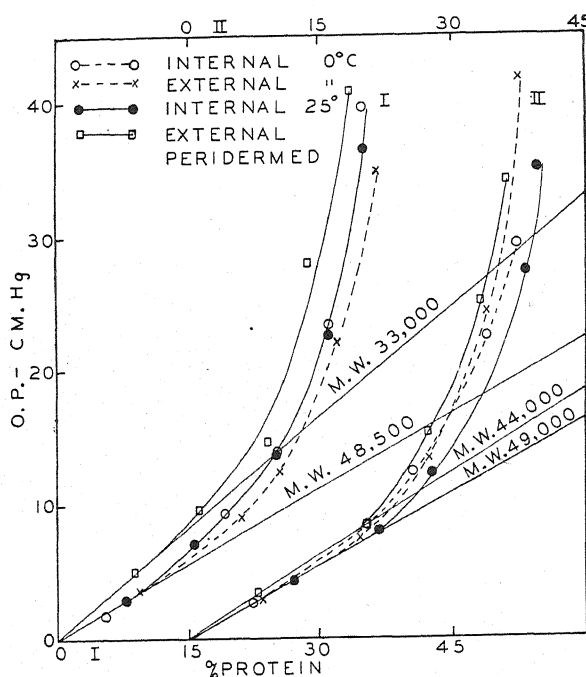


FIG. 6. Osmotic pressure curves for proteins from potato halves at 0° C. and corresponding halves peeled and transferred to 25° C. for 6-7 days to form periderm. Series I, May 1; Series II, May 19.

nal tissues) are identical (1.7). The difference between the proteins from the internal and external tissues of the controls is solely due to a difference in hydration, since the molecular weights are identical.

Any interpretation of these changes in the average properties of the proteins may follow one of two paths. They may be due to a change in the proteins *in situ* or to a transfer of proteins from one region to the other. In all the above tests it was always evident that there were more proteins obtained from the tissues kept at room temperature than from those taken directly from the cold chamber. Quantitative determinations were therefore made (table I).

TABLE II

AVERAGE MOLECULAR WEIGHT IN GRAMS OF PROTEINS FROM POTATO TUBERS

DATE	INTERNAL TISSUES		EXTERNAL TISSUES	
	0° C.	6-7 DAYS AT 25° C.	0° C.	6-7 DAYS AT 25° C.
March 11	46,000	.....	46,000	.....
April 26	.....	.....	.....	53,500
May 1	48,500	48,500	48,500	33,000 (new periderm)
May 20	49,500	49,500	49,500	44,000 (new periderm)
May 11	45,000	45,000	45,000	45,000
May 30	39,000	39,000	39,000	39,000

In both the external and internal tissues a marked increase in proteins occurred. It is quite possible that all the changes in the average properties of the proteins are due to the properties of these newly formed proteins. If so, the actual properties of these new proteins would differ from those of the older proteins to a more marked degree than is indicated by the curves, since these represent averages for both old and new proteins. But the possibility of changes in the old proteins is not excluded. The increase in quantity of proteins on exposure to room temperature is in agreement with the often reported hydrolysis of proteins to products of low molecular weight, at low temperatures (7). It may also explain why a change in average molecular weight sometimes occurs. This may possibly depend on the stage of resynthesis of the proteins.

The remarkable stability of the average molecular weight is shown in table II.

### Discussion

It is, of course, impossible to decide from the above results whether they apply to the proteins as they occur in protoplasm, or whether the methods used markedly alter the properties of the proteins. Cell physiologists have concluded from their observations of living cells that the protoplasm of growing cells is more highly hydrated than that of resting cells (3). The fact that the results reported in this paper agree with this conclusion supports the belief that the isolated proteins retain, at least in part, the properties they possessed while in the protoplasm. The low average molecular weight obtained is perhaps against this view. Indeed, the most remarkable result was the relative stability of the average molecular weight, even when new proteins were being synthesized in considerable quantity. It is obvious, however, that some breakdown of the protoplasmic structure must occur during extraction—even if only at the "haftpunkte" described by FREY-WYSSLING (3). This would not necessarily alter the properties of the individual proteins.

The opposing tendencies of the dormant (internal) as compared with the active (external) tissues are interesting. It is usually stated that increased hydration of proteins occurs at low temperatures (7), yet this has always run counter to the above observations of cell physiologists that hydration increases during growth. The results reported here indicate that these apparently opposite concepts are both tenable, depending on the state of activity of the cells. However, the results reported are too few to permit any generalizations. But the fact that it is possible to detect differences in the properties of the proteins associated with differences in physiological state indicates that this method may prove a valuable one for investigating many problems.

### Summary

1. A method is described for extracting protoplasmic proteins in the undenatured state from potato tubers and for determining their average molecular weight and hydration from osmotic pressure curves.

2. The average molecular weight of the protoplasmic proteins obtained from tubers stored at 0° C. was about 40,000–50,000 grams.

3. The protoplasmic proteins from the internal, dormant tissues were relatively stable in osmotic behavior. On transfer of the tubers from 0° to 25° C. for 6–7 days, the average molecular weight remained constant, and in most cases the hydration decreased slightly.

4. The protoplasmic proteins from the external tissues which were allowed to form new periderm proved more changeable in osmotic behavior. On transfer from 0° to 25° C. either an increase in hydration or a decrease in average molecular weight occurred.

5. Proteins were synthesized in considerable quantity when the tubers were transferred from 0° to 25° C. for 6–7 days. This is in agreement with the numerous reports by other investigators of protein hydrolysis at low temperatures.

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# EFFECT OF INHIBITORS ON THE RESPIRATION AND STORAGE OF COTTONSEED

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AND CATHERINE M. HALL

(WITH THREE FIGURES)

## Introduction

One of the earliest attempts to apply chemical treatment as a means of inhibiting the heating and lipolysis of cottonseed during storage was made by BARROW (4), who used sodium chloride as the effective chemical agent. The application of 5 per cent. of sodium chloride to moist cottonseed had a twofold effect. First, as a result of the high salt concentration outside the seeds, considerable moisture was withdrawn from within. Secondly, small quantities of the salt which diffused into the seed inhibited the biological processes responsible for heating and lipolysis. MALOWAN (17) found that sodium chloride likewise decreased the rate of evolution of carbon dioxide from moist cottonseed and that alcohol, acetic acid, sulphuric acid, and formalin inhibited respiration. He found that certain disinfectants, such as copper sulphate and mercuric chloride, had no effect on the rate of respiration and concluded, therefore, that the carbon dioxide evolution observed under his experimental conditions was caused not by the action of micro-organisms but rather by the action of enzyme systems operating in the seed.

Two of the present authors have reported previously (13) that ammonia and hydrochloric acid vapors inhibit lipolysis of the oil in cottonseed. Subsequent experiments indicated that ammonia treatment also effects the spectrum of extracted cottonseed oil and the heating of seed stored in bulk (1). This treatment of cottonseed with ammonia vapors, although potentially adaptable to commercial practice, was shown in mill-scale tests to be unfeasible as proposed (2).

In the present investigation the effect of ammonia upon a number of properties of both mature and immature cottonseed was measured in order to form a basis for a better understanding of the mechanism of inhibitor action. Since it was also of interest to determine whether the surface sterilization of cottonseed would seriously inhibit respiration and lipolysis or affect the color of the seed oil, a number of chemical agents known to act as inhibitors of microbial growth were also tested.

## Materials and methods

The inhibitors used were ammonia, butyl maleimide, 2'-methyl-1-maleanil, Naccanol NR, Emulsol 607, and Emulsol 607M.

Both butyl maleimide and 2'-methyl-1-maleanil are compounds developed for use as disinfectants (11). The former, a liquid at room temperature,



is able to disinfect pointed steel strips inoculated with *Staphylococcus aureus* when the inoculated strips are suspended above the compound in a closed space for a period of 2 days. Spores of an *Aspergillus* species, when placed on glass slides and exposed to vapors of butyl maleimide in a closed space for 6 days at 50° C. or for 7 days at 25° C., do not germinate. Somewhat related in structure and properties to butyl maleimide, but less effective as a fumigant, is 2'-methyl-1-maleanil, which is a solid at room temperature.

Nacconol NR is the proprietary name for an alkyl aryl sodium sulfonate preparation which contains 36 per cent. organic matter, and possesses wetting and dispersing properties (7). Methods for preparing compounds of this type and descriptions of their chemical composition and structure are given in the patent literature (8, 9, 10). In addition to its detergent properties, Nacconol NR has germicidal properties, inasmuch as 1:500 dilution of the compound will destroy cultures of *Staphylococcus aureus* in 72 hours at 37° C. Nacconol NR is effective also in controlling fungus growth.

Emulsol 607, another type of detergent having germicidal properties (6), is described as the lauric acid ester of colamino formylmethyl pyridinium chloride. It will destroy cultures of *Staphylococcus aureus* in 10 minutes in a dilution of 1:20,000, and cultures of *Eberthella typhosa* in the same length of time in a dilution of 1:15,000. An indication of its fungicidal activity is given by the fact that it kills the spores of *Trichophyton interdigitale* and of *T. rosaceum* in a dilution of 1:20,000.

Emulsol 607M differs from Emulsol 607 in that it is derived from myristic acid and is a more effective bactericidal and fungicidal agent than Emulsol 607. Emulsol 607M was available and was used in the anhydrous form, whereas the Emulsol 607 was used in the form of a 10 per cent. aqueous solution.

Three varieties of cottonseed were used. Two of these, the "Delfos" and "Coker's 200-strain-1" were the same as used in experiments reported in a companion paper (14). The "Delfos" variety was grown in Stoneville, Mississippi in 1942, was fully matured when harvested, and was designated as the 100 series in the paper mentioned. The sample of seeds of the "Coker's 200-strain-1" variety was from bolls which had been harvested before they had properly matured and was previously designated as lot 204. This sample was grown in 1942, in Clemson, South Carolina. The third variety was "Cleve Wilt" grown in Clemson, during the 1943 season. It was fully matured when harvested and is designated as lot 405. Before being used in the inhibition experiments, the seeds were conditioned to a moisture content between 14 and 15 per cent. in the manner described in other papers (13, 14).

Ammonia was introduced by diffusing the gas through the seeds in a closed container for 1 hour. The seeds were then kept in the container (24 hours) to permit them to come to equilibrium with the excess of ammonia that had been adsorbed by the fuzz on the seed. At the end of this period, the pH of the seeds was approximately 8. In some of the experiments

reported, similar supplementary treatments with ammonia were given the seed during the course of the storage period.

Butyl maleimide was introduced by exposing 5 pounds of cottonseed for 1 week to the vapors emanating from 20 ml. of this substance. Exposure was accomplished by placing the seeds and the butyl maleimide in a desiccator which was then evacuated and sealed. At the end of the exposure period, the seeds were removed and placed in an air-tight container.

Inasmuch as Naeconol NR, 2'-methyl-l-maleanil, and Emulsol 607M were solids, treatment with these substances was accomplished by thoroughly mixing the seeds with the powdered material in the following proportions: One hundred grams of Naeconol NR with 5 pounds of seed, 20 gm. of 2'-methyl-l-maleanil with 5 pounds of seed; and 10 gm. of Emulsol 607M with 3 pounds of seed. Treatment with Emulsol 607 was accomplished by spraying 5 pounds of the seeds with 25 ml. of the solution in the form of a fine mist.

The treated seeds were stored in air-tight bottles at room temperature. Samples were removed periodically and examined. The analytical methods used for the determination of respiration intensity, lipolysis rate, pH, and spectrum of the seed oil were the same as described in other papers (1, 3, 13, 14).

#### Effect of inhibitor on respiration

In a series of respiration experiments conducted on the "Delfos" variety (100 series), all the chemical agents mentioned above, except Emulsol 607M, were used.

In its effect on the pattern of respiration, ammonia differed from all of the other chemical agents tested. This is evident from the representative respiration patterns of seeds of approximately the same moisture content which are shown in figure 1. The ammonia-treated seeds exhibited a relatively high initial respiration intensity followed in the intermediate portion of the storage period by low respiration, which began to rise again after 200 days' storage. The seeds which were treated with Naeconol NR, however, exhibited a maximum in respiration intensity during the storage period. Similar respiration patterns were given by the seeds treated with Emulsol 607, butyl maleimide, and 2'-methyl-l-maleanil. Such behavior is typical of most of the respiration experiments reported in a companion paper (14) (cf. figs. 1, 2, and 3).

The relationship between the ammonia concentration (pH) and the respiration intensity is apparent from an examination of table I, in which are given pH values for the ammonia-treated samples during the storage period. Sample 1 (average pH, 8.03) reached a pH of 8 at the end of the initial treatment and was maintained at or above that level for 3 months. This was in part due to the fact that additional ammonia treatments were given during the storage interval, as indicated in the table. After 150 days' storage, the pH of the seeds was slightly below 8, and from then on it dropped steadily to a final value of 7.75. Sample 2 (average pH, 7.91)

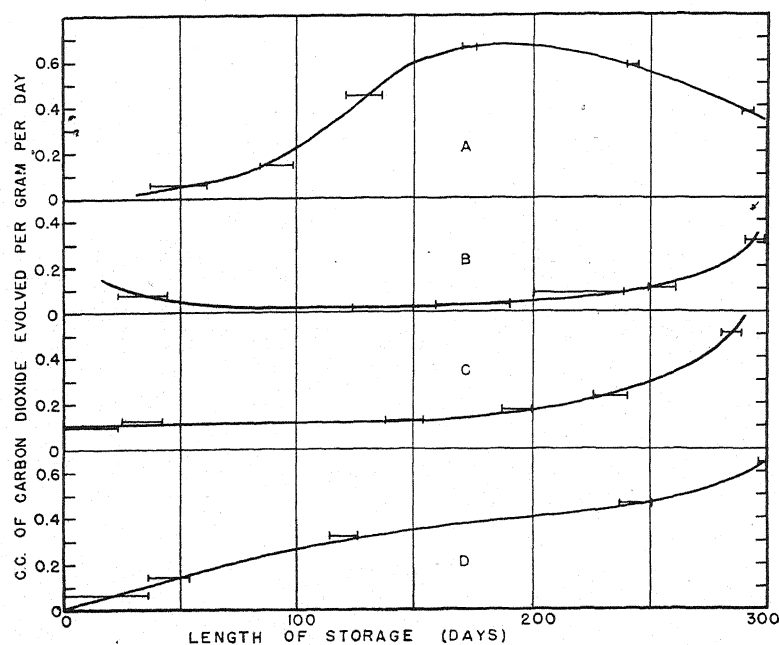


FIG. 1. Effect of inhibitors on the respiration pattern of "Delfos" variety cottonseed (100 series).

Curve	Inhibitor	Moisture content, per cent.
A	Nacconol NR	14.38
B	Ammonia (average pH of 8.03)	14.86
C	Ammonia (average pH of 7.91)	14.76
D	None	14.60

attained a slightly lower pH after the initial treatment than did sample 1, was maintained briefly at a pH above 8, and then was allowed to drop again to lower pH values. The initial stimulation of respiration indicated in

TABLE I  
VARIATION OF pH IN STORED AMMONIA-TREATED COTTONSEED

LENGTH OF STORAGE	SAMPLE 1	SAMPLE 2
<i>days</i>	<i>pH</i>	<i>pH</i>
0	8.00	.....
4	8.05	7.80
25	8.10*	7.85*
32	8.00	7.77
53	8.23*	8.09*
71	8.23*	8.16*
90	8.17	8.12
147	7.94	7.83
218	7.88	7.77
326	7.75	7.79
Average	8.03	7.91

\* These determinations were made following supplementary ammonia treatments.

curve B, figure 1, coincides with the initial heating observed following ammonia treatment (1). Such an initial stimulation of heating also occurred in mill-scale experiments involving the use of ammonia to improve the storage properties of cottonseed (2).

The respiratory patterns of the seeds treated with the inhibitors were integrated in the manner described (14) and their average respiratory intensities (RI) were determined. These values are compared in figure 2

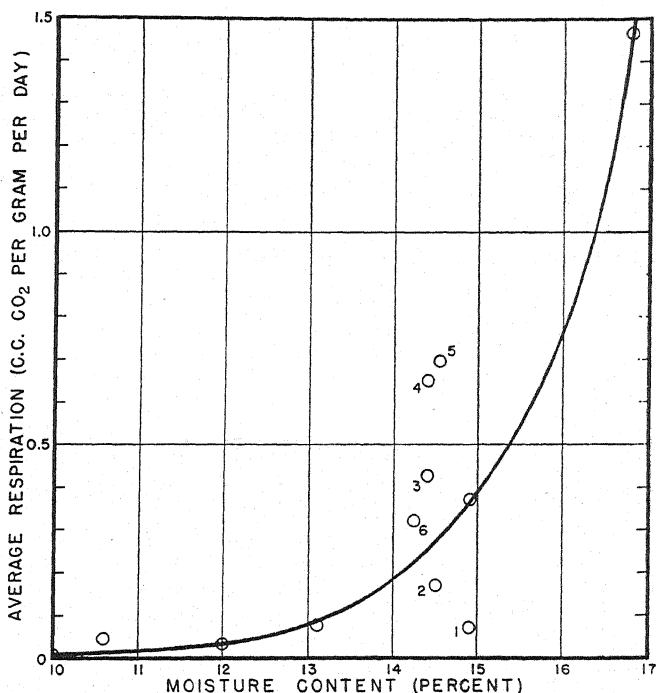


FIG. 2. Effect of inhibitors on the respiratory intensity of "Delfos" variety cottonseed (100 series), the curve representing the respiration of cottonseed in the absence of inhibitors.

1. Ammonia (average pH of 8.03)
2. Ammonia (average pH of 7.91)
3. Nacconol NR
4. Butyl maleimide
5. Emulsol 607
6. 2' methyl-1-maleanil

with those attained on untreated seeds. The curve for untreated seeds shows the effect of moisture content on the respiratory intensity of "Delfos" variety seeds (100 series) as given in figure 4 of (14). It is interesting to note that only treatment with ammonia produced a lower-than-normal average respiratory intensity. The other treatments resulted in an overall stimulation of respiration, regardless of the form in which the fungicide or bactericide was applied.

## Effect of inhibitors on lipolysis rate

Free fatty acid determinations were made on the same lots of seeds used for the respiration measurements, and the lipolysis rate constants (13) were calculated.<sup>2</sup> These are compared in figure 3 with those obtained on normal untreated seeds of the same series. The curve is that for the "Delfos" variety cottonseed (100 series) which was given in figure 1 of a companion paper (15).

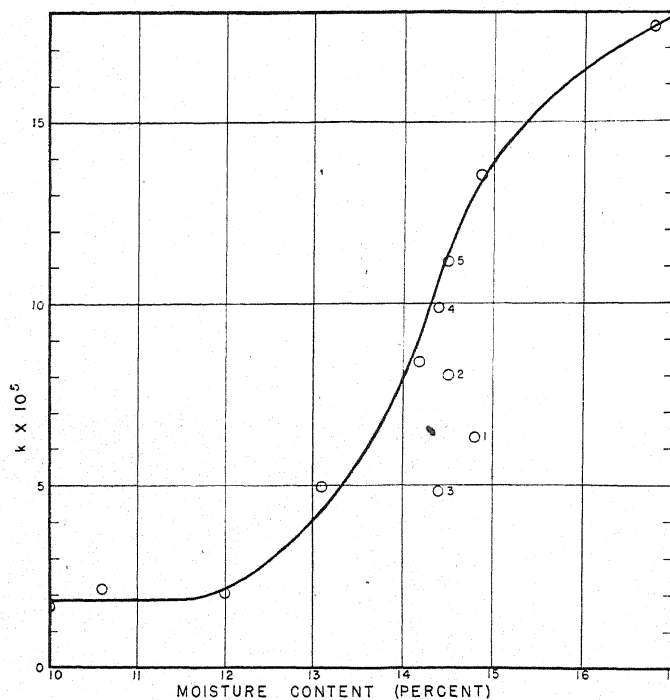


FIG. 3. Effect of inhibitors on lipolysis rate constant,  $k$ , of "Delfos" variety cottonseed (100 series), the curve representing lipolysis of cottonseed in the absence of inhibitors.

1. Ammonia (average pH of 8.03)
2. Ammonia (average pH of 7.91)
3. Nacconol NR
4. Butyl maleimide
5. Emulsol 607

There was no evidence that lipolytic rate was increased by any of the treatments. Emulsol 607 and butyl maleimide had no effect whatsoever. Ammonia had an inhibitory effect similar to that reported in a previous publication (13). Nacconol NR, and to a much lesser extent 2'-methyl-1-maleanil,<sup>1</sup> also reduced the rate of lipolysis.

<sup>1</sup> The curve representing lipolysis as a function of length of storage for the 2'-methyl-1-maleanil-treated seeds was not of the usual autocatalytic type, and, therefore, did not lend itself to the same type of kinetic analysis as did the curves for other normal or treated seeds. Nevertheless, a qualitative examination of data from storage of the seeds

### Comparison of effects of inhibitor on respiration and lipolysis

Effects of the various treatments on respiration and lipolysis are compared in table II. Ammonia treatment produced consistent changes with

TABLE II

EFFECT OF VARIOUS CHEMICAL AGENTS ON THE RESPIRATION INTENSITY AND LIPOLYSIS RATE CONSTANT OF COTTONSEED

CHEMICAL AGENT	RESPIRATION	LIPOLYSIS
	% of normal	% of normal
Ammonia (average pH 8.03) .....	83	52
Ammonia (average pH 7.91) .....	38	29
Naccenol NR .....	169	55
Butyl maleimide .....	261	100
Emulsol 607 .....	185	100
2'-Methyl-1-maleanil .....	145	Inhibited

respect to both processes, whereas the other treatments uniformly stimulated respiration, but either inhibited or had no effect on lipolytic activity.

### Effect of ammonia concentration on lipolysis

A comparison of the results of the two ammonia treatments indicates that the extent of inhibition of lipolysis is a function of the concentration of the ammonia. This fact is clearly demonstrated in the following series of experiments conducted on cottonseed of the "Cleve Wilt" variety. Three-pound lots of seeds were conditioned to a moisture content of approximately 14.2 per cent. and set aside in air-tight bottles. One portion was used as the untreated control. A second portion was subjected to continuous ammonia treatment by placing in the bottle a small beaker full of ammonium carbonate which slowly decomposed and provided a constant supply of ammonia. A third portion was given only one initial ammonia treatment. A fourth portion was given an initial ammonia treatment, and then stored in an air-tight bottle for 30 days. At the end of this period, the ammonia was removed by withdrawing the seed samples from the bottle and spreading them in a current of air until the excess ammonia was dissipated. The seeds were then replaced in the original bottle and stored together with the other three lots.

The results of this experiment, together with those of certain other experiments to be described later, are shown in table III. The free fatty acid content of two of the ammonia-treated samples remained essentially unchanged for the first 100 days of storage. The third ammonia-treated sample (Lot 405eCr) showed a slight increase in free fatty acids (0.30%) during that period. Marked differences among the samples began to develop during the remaining 230 days of storage. The values of the lipolysis

treated with 2'-methyl-1-maleanil indicated that the rate of free fatty acid formation was definitely inhibited. At the end of 340 days' storage, the sample treated with 2'-methyl-1-maleanil developed 12.5 per cent. free fatty acids, as compared to 17.5 per cent. developed by an untreated control of the same moisture content.



rate constant,  $k$ , given in table III represent the overall rates for the entire storage period. It is clear that to realize the maximum effect of inhibition by treatment with ammonia it is necessary to continuously subject the seeds to the action of ammonia vapors.

Nacconol NR was previously found to be the only one of the agents tested which inhibited the lipolytic activity of the cottonseed to a very marked extent. Accordingly, another experiment (405eNv) was planned to determine whether the biological activity of Nacconol NR was due to direct contact with the seeds or to the action of vapors emanating from this material. Three pounds of cottonseed, previously conditioned to 14 per cent. moisture content, were stored in a desiccator above a dish containing 60 gm. of Nacconol NR. In order to compare the activity of solid Emulsol

TABLE III  
EFFECT OF INHIBITORS ON THE LIPOLYSIS RATE OF "CLEVE-WILT"  
VARIETY COTTONSEED

EXPERIMENT	INHIBITOR	MOISTURE CON- TENT	FREE FATTY ACID CONTENT			LIPOLYSIS RATE CON- STANT, $k$	INHIBITION
			ORIGI- NAL	100 DAYS	230 DAYS		
		%	%	%	%	<i>reciprocal of days</i>	%
405 e	None	14.5	0.83	7.14	40.30	$20.4 \times 10^{-5}$	.....
405 eCC	Continuous ammonia	14.7	0.83	0.65	1.78	3.4 "	83.3
405 eC	Single ammonia treatment	14.3	0.83	0.85	7.14	9.6 "	52.9
405 eCr	Excess ammonia removed	14.2	0.83	1.13	28.35	16.7 "	17.9
405 eNv	Nacconol NR vapors	14.0	0.83	1.46	2.75	5.5 "	73.0
405 eE	Emulsol 607M	14.2	0.83	7.21	39.99	20.4 "	None

607M with the previously determined action of an aqueous solution of Emulsol 607, a second experiment (405eE) was conducted by mixing and storing 3 pounds of seed and 10 gm. of Emulsol 607M. From the results given in table III, it is clear that vapors emanating from Nacconol NR have powerful inhibiting properties. On the other hand, Emulsol 607M, a more powerful germicide than Emulsol 607, exhibits no inhibiting effect on the lipolysis rate.

The discovery that vapors from Nacconol NR preparations inhibit the formation of free fatty acids in cottonseed may be of great practical significance to the cottonseed industry. Preliminary mill-scale experiments on the use of this material to improve the storage properties of cottonseed and flaxseed have indicated that improvement may, in fact, be achieved (2). Further investigations designed to determine the nature and mode of action of the biologically active vapors are in progress.

#### Effect of inhibitors on the spectrum of extracted oils

The observation, reported in a previous publication (1), that ammonia treatment of cottonseed reduces the color of the solvent-extracted oils was

confirmed and extended in the present investigation. It was found, however, that the inhibitors other than ammonia which were tested did not affect the spectrum of the oil. In table IV are shown the results of the effects of various inhibitors on the light absorption of the solvent-extracted cottonseed oils at 560 m $\mu$ , the wavelength of maximum light absorption of gossypurpurin (5). It can be seen that the concentration of ammonia influences the rate at which the absorption at 560 m $\mu$  is reduced. A similar effect was observed when the light absorption of the oil from ammonia-

TABLE IV

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 m $\mu$  OF THE OILS FROM "DELPHOS" VARIETY COTTONSEED (100 SERIES) AS AFFECTED BY VARIOUS INHIBITORS

INHIBITOR	LENGTH OF STORAGE	LOG I <sub>0</sub> /I*	INHIBITOR	LENGTH OF STORAGE	LOG I <sub>0</sub> /I
	<i>days</i>			<i>days</i>	
None	29	0.018	Ammonia (ave. pH of 8.03)	32	0.012
	120	0.017		90	0.006
	156	0.011		147	0.004
	337	0.028		326	0.007
Ammonia (ave. pH of 7.91)	32	0.014	Nacconol NR	29	0.018
	90	0.009		71	0.018
	147	0.007		157	0.012
	326	0.006		337	0.038
Emulsol 607	29	0.018	Butyl maleimide	29	0.018
	71	0.018		71	0.021
	157	0.012		158	0.018
	337	0.013		337	0.017
2'-Methyl-1-maleanil	29	0.018			
	71	0.017			
	158	0.012			
	337	0.017			

\* All absorption data are calculated for solutions in carbon tetrachloride containing one ml. of oil in a total of 50 ml. of solution. The absorption cell length was 13 mm.

treated seeds at 360 m $\mu$ , the wavelength of maximum light absorption of gossypol, was compared to that of the oil from the untreated control.

#### Effect of ammonia on immature cottonseed

In the course of previous work (14, 15), it was noted that an immature sample of seeds ("Coker's 200, strain 1") behaved entirely differently, with respect to both lipolysis rate pattern and respiration intensity, from all of the other samples investigated. The present experiments show that this difference in behavior is also reflected in the reaction of the seeds to ammonia treatment.

When immature seeds were treated with ammonia to raise the pH of the sample to 8.3, the overall respiratory intensity was greatly reduced, in spite of a marked stimulation of respiration immediately following exposure of the seeds to ammonia vapors. Thus, the overall respiratory intensity of untreated immature seeds (sample 204b) was 0.14 cc. of carbon dioxide

per gram per day, compared to 0.0027 cc. for comparable ammonia-treated seed (sample 204c). The moisture contents of the two lots were practically the same; *i.e.*, 12.5 per cent. for sample 204b and 12.1 per cent. for sample 204c. The decrease in respiratory intensity was much greater than that usually encountered when mature seeds were treated with ammonia. For example, mature ammonia-treated seeds ("Delfos" variety) which were kept at an average pH of 8.03 had a respiratory intensity of 0.07 cc. of carbon dioxide per gram per day compared to a value of 0.37 for the untreated control lot of the same moisture content. This represents an inhibition of 81 per cent. as compared to an inhibition of 98 per cent. occasioned by similar treatment of immature seeds. In addition to the quantitative differences in respiration intensity between the immature and normal seeds, the respiratory quotient of the ammonia-treated immature seeds (sample 204c) was found to be 0.88 as compared to 0.95 for untreated immature seed (sample 204b). On the other hand, ammonia was found to exert no effect on the respiratory quotient of mature seeds.

TABLE V

CHANGES IN LIGHT ABSORPTION AT 360  $m\mu$  AND 560  $m\mu$  OF THE OILS EXTRACTED FROM IMMATURE SEEDS (SAMPLE 204) DURING STORAGE

SAMPLE								
204A			204B			204C		
TREATMENT								
NONE			MOISTURE CONTENT REDUCED			AMMONIA		
MOISTURE CONTENT								
13.7 PER CENT.			* 12.5 PER CENT.			12.1 PER CENT.		
LENGTH OF STORAGE	Log I <sub>0</sub> /I 360 mμ	Log I <sub>0</sub> /I 560 mμ	LENGTH OF STORAGE	Log I <sub>0</sub> /I 360 mμ	Log I <sub>0</sub> /I 560 mμ	LENGTH OF STORAGE	Log I <sub>0</sub> /I 360 mμ	Log I <sub>0</sub> /I 560 mμ
<i>days</i>			<i>days</i>			<i>days</i>		
0	12.60	0.030	0	8.24	0.041	0	3.19	0.078
41	6.74	0.034	31	4.42	0.031	31	2.59	0.062
69	4.72	0.036	67	3.95	0.033	53	2.16	0.062
112	3.51	0.041	110	2.94	0.048	87	1.96	0.077
142	3.44	0.051	140	3.78	0.048	138	0.74	0.079
212	4.46	0.070	210	6.43	0.074			

Whereas the respiration of immature cottonseed was inhibited by ammonia, this treatment had the opposite effect on the rate of lipolysis. As was shown in figure 5 of a companion paper (15), the lipolysis patterns of immature and mature seeds differed fundamentally; the results obtained on immature seeds could not, therefore, be subjected to the same type of mathematical analysis as was used in the case of the results obtained on mature seeds. Both the ammonia-treated and untreated immature seeds gave a similar type of lipolysis pattern. Ammonia stimulated lipolysis of immature seeds, whereas it invariably inhibited lipolysis of the mature seeds. After 200 days of storage, the untreated immature seeds with a 12.5 per

cent. moisture content developed 16 per cent. free fatty acids; but after the same period, the ammonia-treated immature seeds with a 12.1 per cent. moisture content developed 24.5 per cent. free fatty acids.

The effects of ammonia treatment on the absorption spectra of oils extracted from immature seeds and on those extracted from mature seeds were also profoundly different. In table V are given the light absorptions at 360  $m\mu$  and 560  $m\mu$  of the oils from the three lots of immature seeds as a function of length of storage. In table VI is given a summary of all of the types of spectral changes encountered. The untreated immature seeds exhibited at 560  $m\mu$  a pattern of change which was not of the same nature found for the mature seeds. Thus, except for an initial drop, the oil from sample 204b exhibited a steady rise in light absorption as the length of the storage period increased. Sample 204a exhibited a steady rise in light ab-

TABLE VI

EFFECT OF STORAGE ON THE PATTERN OF CHANGES IN LIGHT ABSORPTION OF THE OILS EXTRACTED FROM UNTREATED AND AMMONIA-TREATED, MATURE AND IMMATURE COTTONSEED

VARIETY	TYPE	TREATMENT	PATTERN OF CHANGES AT 360 $m\mu$	PATTERN OF CHANGES AT 560 $m\mu$
"Delfos"	Mature	None Ammonia	Reversal of change Gradual drop	Reversal of change Gradual drop
"Coker's-200 strain 1"	Immature	None Ammonia	Reversal of change Gradual drop	Steady rise No change

sorption from the very beginning of the storage period. On the other hand, the ammonia-treated immature seeds (sample 204c) had an initially higher light absorption in this region, and this absorption did not vary throughout the storage period. The ammonia treatment of mature seeds has been previously shown to lower gradually the light absorption of the extracted oil in the same region (15).

With respect to absorption in the 360  $m\mu$  region, the two untreated immature seed samples exhibited a reversal of change, whereas the ammonia-treated immature sample showed the steady decrease which is characteristic of treated mature seeds. Thus ammonia treatment has a similar effect on the spectra of the oils of both mature and immature seeds in the 360  $m\mu$  region.

The data in table VI confirm the observation made by BOATNER (5) that the light absorptions at 360  $m\mu$  and at 560  $m\mu$  are not due to the same pigment systems. In the mature seeds, these pigments are affected similarly by both storage and ammonia treatment. In immature seeds, however, these two pigment systems behave independently of each other.

#### Discussion

The initial stimulation of respiration, observed when cottonseed is treated with ammonia, is common to many types of reactions involving poisons and

drugs. IRVING (12) observed that the respiration of leaves was initially stimulated by exposure to chloroform vapors; when the concentration of chloroform was raised sufficiently, this initial stimulation was rapidly followed by an inhibition of respiration. The initially higher respiration which resulted from the treatment of seeds with ammonia was of such a short duration that the overall effect of this treatment was a decrease in the respiratory intensity of the seeds. It is conceivable that had the concentration of ammonia in the seeds been kept sufficiently low throughout the storage period, the stimulation would have been maintained throughout, and the net effect would have been an increase in respiratory intensity over that of the normal untreated seeds.

An increase in respiratory intensity is what actually occurred in the seeds treated with Nacconol NR, Emulsol 607, butyl maleimide, and 2'-methyl-1-maleanil. In every case, there was a stimulation of respiration which persisted throughout the storage period and resulted in a higher than normal value for respiratory intensity. In large-scale storage experiments, however, it has been possible to increase the concentration of inhibitor (Nacconol NR vapors) to such an extent that the initial stimulation of biological activity is soon replaced by marked inhibition (2).

It is significant that the inhibitions of respiration and of lipolysis are not strictly parallel phenomena. That it is possible to inhibit one activity and stimulate the other in the same lot of seeds was shown most clearly by the effect of the Nacconol NR treatment. Thus, it would seem that the processes of respiration and lipolysis are not functionally related in resting seeds; one system may be inhibited under conditions that will stimulate the other. The system involved in lipolysis is apparently more easily inhibited than is that involved in respiration; this is evident in the results obtained on mature seeds where it can be seen there are a number of chemical treatments which inhibit lipolysis but stimulate respiration.

The lack of parallelism between respiration and lipolysis suggests a possible method for distinguishing between deterioration caused by the bacteria and molds that are associated with the seeds and that resulting from the operation of the enzyme systems of the seed themselves. It has been tacitly assumed in many investigations of the deterioration of seeds during storage, that the micro-organisms associated with the seed are the primary cause of deterioration. Thus RAMSTAD and GEDDES (19) have suggested that most of the heating that takes place in moist soybeans is due to the action of micro-organisms. Similarly, deterioration in flaxseed has been attributed to microbial activity (16, 20). Even though surface sterilization of the seeds rarely affected their rate of respiration or heating, results obtained by use of such procedures could not be offered as clear-cut evidence that the respiration was independent of bacterial action, because surface sterilization obviously could have no effect on the micro-organisms present in the interior of the seeds. Any attempt to achieve sterilization within the seeds would, of course, yield inconclusive results because biological systems



of the seeds would also be affected by such a sterilization procedure. Yet, if micro-organisms were the sole cause of respiration, heating, and lipolysis obtained in stored seed, it would be expected that the inhibition of the growth of such organisms would affect all of these processes in a similar manner. On the other hand, if most of the deteriorative action were due to systems within the seeds themselves, it is conceivable that certain biological functions would be more susceptible to inhibition than others. Therefore, when these criteria are applied to the behavior of cottonseed and are considered in conjunction with the evidence presented in this and the preceding papers, it becomes abundantly clear that the bulk of the deteriorative processes in stored cottonseed, such as respiration, heating, and lipolysis, are due to the activity of seed enzyme systems.

The evidence upon which this conclusion is based may be summarized as follows:

1. Surface sterilization has no effect on the respiration of cottonseed. Despite the variety of the fungicides and bactericides used, none has been found to inhibit respiration. These results confirm those of MALOWAN (17) who showed that solutions of mercuric chloride or copper sulfate had no inhibitory effect on the heating of cottonseed. Obviously, therefore, surface growth of micro-organisms does not materially contribute to the heating or respiration of the seeds.

2. Nacconol NR has a differential effect on the respiration and lipolysis of cottonseed; under the experimental conditions that were generally used, respiration was stimulated, whereas lipolysis was markedly inhibited. With the exception of the ammonia treatment, the chemical treatments applied stimulated respiration, but they had no such effect on lipolysis.

3. Under certain conditions, it was possible to effect by chemical treatment an inhibition of respiration but a stimulation of lipolysis. Thus, the treatment of immature seeds with ammonia resulted in greatly reduced respiration, but increased lipolysis.

4. Every aspect of the behavior of immature seeds (the 204 series) differs from that of mature seeds; respiration is much higher than normal; the lipolysis pattern is different; spectral changes and the reaction to inhibitors differ markedly from those of normal seed. It is inconceivable that the microbial population of immature cottonseed should differ so greatly from that of mature cottonseed as to change both the degree and pattern of respiration, lipolysis, and spectral changes.

It is possible to conclude, therefore, that under the experimental conditions of temperature and moisture reported in this series of publications, the major portion of the biological activity observed results from the activity of seed enzyme systems. The initial stages of deterioration including the initial heating caused by respiration (1, 2, 18), which occur in cottonseed during commercial bulk storage, are almost certainly a result of seed enzymatic activity. Unquestionably, the microbial growth which takes place during storage may reach such proportions as to contribute to



the apparent biological activity of the seeds. But this microbial effect is secondary in nature and is evident only after prolonged storage.

It follows from these conclusions that, with the exception of seeds having excessively high moisture content, any attempt to reduce the biological activity of stored cottonseed must concern itself first with the inhibition of the seed enzyme systems.

### Summary

1. Treatment of cottonseed with ammonia was found to inhibit the respiration and lipolysis of mature seeds and reduce the light absorption of the extracted oil at 360 m $\mu$ .

2. Similar treatment of immature cottonseed was found to inhibit respiration, but to stimulate lipolysis. Light absorption of the extracted oil at 560 m $\mu$  was increased, whereas the oil from ammonia-treated mature seeds exhibited decreased absorption at this wave length.

3. The vapors of Nacconol NR were found to inhibit lipolysis in cottonseed under conditions where there was a stimulation of respiration. Treatment of cottonseed with 2'-methyl-1-maleanil yielded similar results.

4. Fungicides and germicides such as Emulsol 607M, Emulsol 607, and butyl-maleimide had no effect on the lipolysis rate of stored cottonseed; the last two substances stimulated respiration.

5. Evidence has been presented to demonstrate that most of the deterioration which occurs in stored cottonseed is due to the action of enzymes in the seeds rather than to microbial activity.

The authors wish to thank G. W. IRVING, JR. and F. G. DOLLEAR for many helpful suggestions. The samples of Nacconol NR, butyl maleimide, and 2'-methyl-1-maleanil were kindly furnished by LAWRENCE H. FLEET, of the National Aniline Division of the Allied Chemical and Dye Corporation, and the samples of Emulsol 607 and Emulsol 607M were furnished through the courtesy of ALBERT K. EPSTEIN of the Emulsol Corporation.

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## EFFECT OF 2,4-DICHLOROPHENOXY ACETIC ACID ON THE RIPENING OF BARTLETT PEARS<sup>1</sup>

ELMER HANSEN

(WITH ONE FIGURE)

It has long been known that ethylene and certain other unsaturated hydrocarbon gases have the property of stimulating the ripening of various fruits. Recent data indicate that similar effects can be produced by several of the synthetic plant-growth hormones. Thus, the maturation and ripening of certain varieties of apples and pears have been observed to be directly influenced by naphthalene acetic acid when used as a spray to retard harvest-drop (1, 2). MITCHELL and MARTH (6) found that the time required for the ripening of green bananas and freshly-harvested apples and pears was reduced by treatment with 2,4-dichlorophenoxy acetic acid. These treatments were applied to fruits in the pre-climacteric stage when the quantity of ethylene metabolized by the tissues is known to be extremely small (4, 5). No data are available to indicate their effect in the presence of physiologically active quantities of ethylene, such as occur in post-mature and storage fruits. In the present study, the comparative effects of 2,4-dichlorophenoxy acetic acid on the respiration and ripening of Bartlett pears, which vary in stage of maturity and capacity for ethylene production, are considered.

### Materials and methods

Two collections of pears were made from a single tree located at the Experiment Station orchard near Corvallis. The first sample was picked on August 14 and represented fruit in an immature stage of development. The second sample was collected two weeks later when the fruit was at the optimum stage of maturity for commercial use. A portion of this collection was stored at 31° F. for five weeks prior to treatment.

The method used in treating pears with 2,4-dichlorophenoxy acetic acid consisted of immersing the fruit in an aqueous solution containing 1000 p.p.m. of the reagent and one per cent. Carbowax. In some experiments other dilutions were used as indicated later. The treated fruits were allowed to dry before transferring to the respiration chambers.

The rates of carbon dioxide and ethylene production were determined by the methods described previously (3).

### Results and discussion

#### IMMATURE FRUIT

Immediately after picking, the pears were divided into four uniform lots, each consisting of 15 fruits. Lot 1 was treated with 2,4-D and then con-

<sup>1</sup> Published as Technical Paper 478 with the approval of the Director of the Oregon Agricultural Experiment Station, Oregon State College, Corvallis, Oregon. Contribution of the Department of Horticulture.

fined in a desiccator in order to retain the ethylene emanating from the fruit. Lot 2 was untreated but similarly confined during the course of the experi-

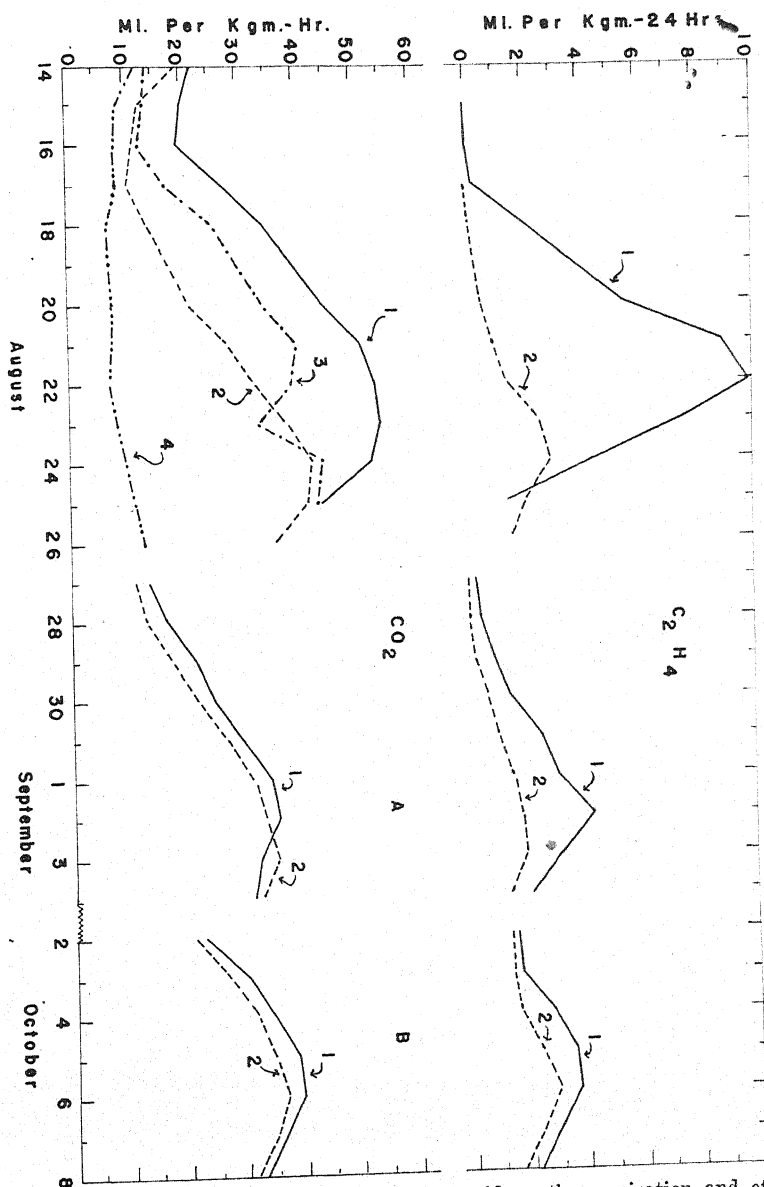


FIG. 1. Effect of 2,4-dichlorophenoxy acetic acid on the respiration and ethylene production of Bartlett pears. 1, treated-unaerated; 2, untreated-unaerated; 3, treated-aerated; 4, untreated-aerated. A, mature fruit ripened before storage; B, mature fruit ripened after 5 weeks' storage.

ment. Lot 3 was treated with the acid, then placed in a desiccator which was aerated with a constant flow of fresh air (20 liters per hour) in order

to remove ethylene from the storage atmosphere. Lot 4 was untreated and constantly aerated. The rates of carbon dioxide production were determined daily on all lots, while ethylene determinations were made only on fruit kept in the closed containers.

The amount of ethylene produced by the immature pears at the beginning of the experiment (fig. 1) was below the chemically determinable minimum, and when constant aeration was provided, as in Lot 4, the fruit remained in the preclimacteric stage and failed to ripen during the course of the experiment. When ethylene was allowed to accumulate in the storage atmosphere, as in Lot 2, respiratory activity increased and the fruit was fully ripe at the peak of the climacteric. Similar stimulation occurred in the samples treated with 2,4-D. The treated fruit, which was unaerated (Lot 1), however, attained a higher maximum rate of respiration and ripened one day sooner than the treated fruit, which was aerated (Lot 3). These data indicate that 2,4-D and ethylene in combination have a greater influence on respiration and ripening than either reagent has when used separately.

Treatment with 2,4-D greatly increased the rate of ethylene formation by the fruit. In the treated sample, ethylene production attained a maximum value of 10.06 ml. as compared to 2.84 ml. per kilogram per 24 hours in the untreated fruit. This represents a 3.6-fold increase due to treatment, and since there was only a 1.3-fold increase in rate of respiration in the same fruit, the production of ethylene and carbon dioxide do not appear to have been equally influenced by 2,4-D.

#### MATURE FRUIT

The rates of ethylene and carbon dioxide production were determined on treated and untreated pears before and after five weeks' storage at 31° F. In addition, the rates of ripening were determined on separate lots of fruit which were treated with 2,4-D in concentrations of 10, 50, 100, 250, 500 and 1000 p.p.m. then kept in well-ventilated room maintained at a temperature of 65-70° F.

The initial rate of ethylene production in the mature pears before storage was less than 0.001 ml. per kilogram per 24 hours, and during ripening increased to 1.86 ml. and 4.31 ml. in the untreated and treated lots, respectively (fig. 1). There was very little difference in the rate of respiration between the two lots, although the peak of the climacteric occurred one day sooner in the treated fruit. As indicated by pressure tests, all lots of fruit treated with 2,4-D in concentrations of 50 p.p.m. and higher ripened in seven days, while the untreated lot ripened in nine days. The green color of the fruit treated with the acid in concentrations of 100 p.p.m. and higher failed to disappear uniformly, resulting in the development of a yellow-green, mottled appearance. This condition was not evident on fruit treated only with one per cent. Carbowax, nor in fruit treated with 2,4-D after five weeks of cold storage.

The pears which had been kept in cold storage for five weeks were still

firm and green and differed but little in outward appearance from freshly-harvested fruit. The data show, however, that the respiratory activity and the capacity for ethylene production were much greater than before storage, and it is evident that the climacteric was in progress at time of treatment. Both carbon dioxide and ethylene production tended to be slightly higher in the treated fruit, but the peak of the climacteric in both treated and untreated lots occurred on the same day. All lots of fruit, including the controls as well as those treated with varying concentrations of 2,4-D were fully ripe on the sixth day. According to these data, the time required for the ripening of Bartlett pears is unaffected by 2,4-dichlorophenoxy acetic acid when treatment is applied after the climacteric has been initiated.

### Summary

Aqueous solutions of 2,4-dichlorophenoxy acetic acid in 1 per cent. Carbowax were applied to Bartlett pears at different stages of maturity and their effect on ripening, respiration and ethylene production determined.

Treatment of premature pears resulted in an increase in the rates of ripening, respiration, and ethylene production. The maximum values for carbon dioxide and ethylene production were 1.3 and 3.6 times greater, respectively, in the treated than in the untreated fruits. Ethylene and 2,4-dichlorophenoxy acetic acid in combination appeared to have a greater effect on ripening, respiration and ethylene production than either reagent used separately.

Mature pears treated shortly after harvest showed higher rates of respiration and ethylene production and ripened two days sooner than similar untreated fruit. The time required for the ripening of mature pears which had been stored at 31° F. for five weeks was not reduced by treatment with 2,4-dichlorophenoxy acetic acid. The rates of carbon dioxide and ethylene production, however, tended to be higher in the treated fruits.

The author is indebted to DR. PAUL C. MARTH, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agriculture Research Administration, U. S. Department of Agriculture, Beltsville, Maryland, for furnishing the 2,4-dichlorophenoxy acetic acid solutions in Carbowax.

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## NOTES

**Colloids, Their Properties and Applications.**—A. G. WARD. Inter-science Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 133 pages. \$1.75.

This small book presents a non-technical, introductory account of the physics and chemistry of colloids with many references to their applications in physics, engineering, geology, and biology. Though there is relatively little emphasis given to colloids in biology, the book is nevertheless of value to biologists because of the discussion on general characteristics of colloids which comprises the largest portion of the text. A brief bibliography lists the standard reference books on colloids and physical chemistry. The book will be of interest to students wishing to obtain a concise, non-mathematical overview of the colloidal state and common colloidal systems. The text is liberally illustrated, clear, and concise. A subject index permits rapid reference to specific topics.

**Currents in Biochemical Research.**—Edited by D. E. GREEN. Inter-science Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 486 pages. \$5.00.

Thirty-one essays are presented in this volume outlining the present course of biochemical research as it relates to agriculture, medicine, and social problems. Each unit is written by a well-qualified specialist in a style and terminology devoid of abstruse technicalities. The book is in the nature of a concise digest aimed to acquaint scientists with trends, techniques, and needs in various phases of biochemical study. Each section carries a bibliography of the most important recent researches upon which the discussions are based. References do not include titles except for books cited. There is no general index to the volume itself.

Essays of special interest to plant scientists include: The Gene and Biochemistry, Viruses, Photosynthesis, Nutrition of Plants, The Bacterial Cell, Enzymes, Enzymatic Carbon Dioxide Assimilation, Oxidation and Reductions, Isotope Techniques, and Plant Hormones. The book serves an important purpose in permitting a rapid acquaintance with an extremely broad area of research without the laborious effort of consulting the original literature of the many subjects discussed. The essays are lucid and accurate. The authors, editor, and publishers deserve commendation for their preparation of a most well-selected collection of biochemical essays which will be welcomed by chemists, physicists, and biologists as a means of maintaining contact with developments in extremely diversified fields of biochemical research.

**Forest Soils and Forest Growth.**—S. A. WILDE. Volume XVIII of A New Series of Plant Science Books, 1946. Chronica Botanica Co., Waltham 54, Massachusetts and G. E. Stechert and Co., 31 E. Tenth St., New York, N. Y. 241 pages. \$5.00.

This book is something of a pioneer in its field. The author discusses soils from the standpoint of composition of forest stands and their effect upon individual trees, especially in relation to morphological pattern, growth rate, quality of wood, reproductive vigor, and resistance to adverse biotic and climatic conditions. Soils are viewed as carriers of definite floristic associations, as media for growth of nursery stock or forest plantations and as dynamic systems varying in the reaction to different forms of silvicultural treatment.

Several chapters are devoted to the genesis and genetical classification of soils in relation to silviculture followed by descriptions of the physical, chemical, and biotic properties of forest soils. There is an excellent chapter on the biological structure of forest cover and its relation to environment. Other chapters deal with soils in relation to forest management and to sound silvicultural practice. The book includes a very comprehensive bibliography as well as separate author and subject indexes. The author has performed an excellent service in correlating and interpreting the research of soil science, ecology, and physiology in relation to the growth and forestry procedures. The format of the book and numerous illustrations facilitate reading and comprehension. The personal background of the author has permitted him to correlate especially well the European and American researches in relation to forest soils.

**Enzymes and Their Rôle in Wheat Technology.**—Volume I, Monograph Series, edited by J. ANSEL ANDERSON. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 371 pages. \$4.50.

This is the first of a series of monographs to be published by the American Association of Cereal Chemists primarily as a service to its members, but also to biologists in general. The introductory chapter, devoted to the general chemistry of enzymes, is followed by twin discussions of carbohydrase, protease, esterase, oxidase, and fermentive enzymes. The first of the two essays on each topic is a general discussion of existing knowledge of a particular group of enzymes, followed by a section on the technology of the same group, especially in relation to the use of cereals. Each section is written by a well-qualified expert and contains a very complete bibliography including subject titles as well as author and journal citations.

The text is abundantly illustrated and the format permits easy reading. Separate author and subject indexes facilitate rapid reference, adding materially to the usefulness of the book. The editor and authors have made an excellent choice of materials and integrated an extremely diversified mass of data into a logical and coherent account. The general discussions of particular groups of enzymes make this monographic review of interest to all biologists.

**Annual Review of Biochemistry.**—Volume XV, edited by JAMES MURRAY LUCK. Annual Reviews, Inc., Stanford University, California. 687 pages. \$5.00.

Contained in this volume are 21 separate sections of which the following are of particular interest to plant scientists: Plant Carbohydrates by S. Peat; Growth Factors for Microorganisms by E. E. Snell; Photosynthesis by C. S. French; The Respiration of Plants by W. O. James; Biochemistry of Yeast by C. Neuberg; Bacterial Metabolism by H. A. Barker and M. Doudoroff; The Vitamins by R. A. Dutcher and N. B. Guarrant; Organic Insecticides by W. M. Hoskins and R. Craig; The Viruses by N. W. Pirie; Biological Oxidations and Reductions by K. A. C. Elliott; Non-oxidative Enzymes by A. M. Wynne; and The Chemistry of the Steroids by T. Reichstein and H. Reich. Each section carries a comprehensive bibliography. The volume has separate and very complete subject and author indexes.

**Advances in Enzymology.**—Volume VI, edited by F. F. NORD. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 563 pages. \$6.50.

This volume is now ready and includes sections on the following subjects: Bacterial Amino Acid Decarboxylases by E. F. Gale; Enzyme Problems in Relation to Chemotherapy by M. G. Sevag; Biological Antagonisms between Structurally Related Compounds by D. W. Woolley; Adenosine-triphosphatase Properties of Myosin by V. A. Engelhardt; States of Altered Metabolism in Diseases of Muscle by C. L. Hoagland; Acetyl Phosphate by Fritz Lipmann; Microbial Assimilations by C. E. Clifton; Chemical Changes in Harvested Tobacco Leaf by W. G. Frankenburg; Action of the Amylases by R. H. Hopkins; Amylases of Wheat and Their Significance in Milling and Baking Technology by W. F. Geddes; and Tocopherol Interrelationships by K. C. D. Hickman and P. L. Harris.

In harmony with the policy of *Advances in Enzymology*, the foregoing critical reports cover borderline subjects between physiology, microbiology, chemistry and physical chemistry. Each section contains a complete bibliography. This volume (VI) carries separate cumulative subject and author indexes for volumes I to VI as well as the usual indexes to volume VI.

**The Periodic Partial Failures of American Cottons in the Punjab: Their Causes and Remedies.**—R. H. DASTUR. Indian Central Cotton Committee, Bombay. 144 pages. \$2.00.

Dastur has assembled the major results on this topic as they have appeared in the *Indian Journal of Agriculture Science* and other periodicals. The importance to India of the replacement of short staple indigenous cottons by American varieties makes the publication an extremely useful reference. Discovery of the fact that cotton failure or Tirak disease was correlated either with light, sandy soils low in nitrogen, or with sub-soils of high alkalinity was not only of practical importance but resulted in perfection

of new methods of cotton planting, culture, and fertilization which largely overcome otherwise frequent crop failures.

The author traces the history of American cottons in India, procedures used in identification and experimental reproduction of physiological failures, as well as of various successful methods of avoiding losses. In addition to the value of the data to cotton growers, the book provides excellent examples of the application of purely scientific methods in isolation and identification of salient factors involved in complicated plant responses. The general method of attack and details of procedure are of interest to agronomists and physiologists in general in relation to problems connected with practical crop production. Data are well organized and clearly presented. A selected bibliography on cotton is included.

**The Naturalists' Directory.**—Current edition September, 1946, Salem, Massachusetts. \$3.00 postpaid. The directory contains names, addresses, and special subjects of study of professional and amateur naturalists throughout the world. It has been published regularly for sixty years.

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